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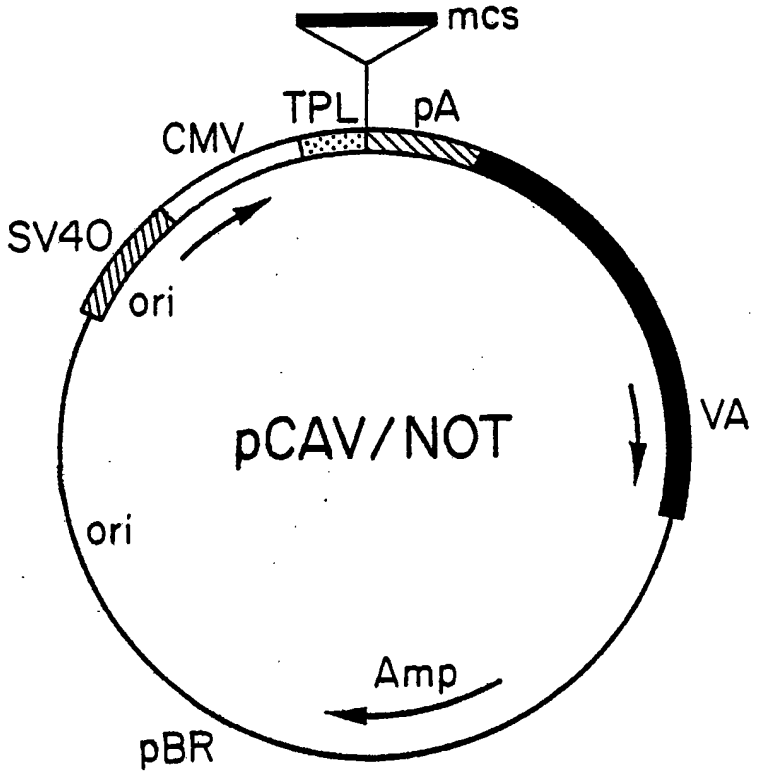

(57) Abstract

Mammalian Interleukin-4 receptor proteins, DNAs and expression vectors encoding mammalian IL-4 receptors, and processes for producing mammalian IL-4 receptors as products of cell culture, are disclosed.

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TITLE

Interleukin-4 Receptors

BACKGROUND OF THE INVENTION

This is a continuation-in-part of application Serial No. 07/326,156, filed March 20, 1989, which is a continuation-in-part of Serial No. 07/319,438, filed March 2, 1989, now abandoned, which is a continuation-in-part of Serial No. 07/265,047, filed October 31, 1988, now abandoned.

The present invention relates generally to cytokine receptors and, more specifically, to interleukin-4 receptors.

Interleukin-4 (IL-4, also known as B cell stimulating factor, or BSF-1) was originally characterized by its ability to stimulate the proliferation of B cells in response to low concentrations of antibodies directed to surface immunoglobulin. More recently, IL-4 has been shown to possess a far broader spectrum of biological activities, including growth co-stimulation of T cells, mast cells, granulocytes, megakaryocytes, and erythrocytes. In addition, IL-4 stimulates the proliferation of several IL-2- and IL-3-dependent cell lines, induces the expression of class II major histocompatibility complex molecules on resting B cells, and enhances the secretion of IgE and IgG1 isotypes by stimulated B cells. Both murine and human IL-4 have been definitively characterized by recombinant DNA technology and by purification to homogeneity of the natural murine protein (Yokota et al., *Proc. Natl. Acad. Sci. USA 83*:5894, 1986; Noma et al., *Nature 319*:640, 1986; and Grabstein et al., *J. Exp. Med. 163*:1405, 1986).

The biological activities of IL-4 are mediated by specific cell surface receptors for IL-4 which are expressed on primary cells and *in vitro* cell lines of mammalian origin. IL-4 binds to the receptor, which then transduces a biological signal to various immune effector cells. Purified IL-4 receptor (IL-4R) compositions will therefore be useful in diagnostic assays for IL-4 or IL-4 receptor, and in raising antibodies to IL-4 receptor for use in diagnosis or therapy. In addition, purified IL-4 receptor compositions may be used directly in therapy to bind or scavenge IL-4, providing a means for regulating the biological activities of this cytokine.

Although IL-4 has been extensively characterized, little progress has been made in characterizing its receptor. Numerous studies documenting the existence of an IL-4 receptor on a wide range of cell types have been published; however, structural characterization has been limited to estimates of the molecular weight of the protein as determined by SDS-PAGE analysis of covalent complexes formed by chemical cross-linking between the receptor and radiolabeled IL-4 molecules. Ohara et al. (*Nature 325*:537, 1987) and Park et al. (*Proc. Natl. Acad. Sci. USA 84*:1669, 1987) first established the presence of an IL-4 receptor using radioiodinated recombinant murine IL-4 to bind a high affinity receptor expressed in low numbers on B and T lymphocytes and a wide range of cells of the hematopoietic lineage. By affinity cross-linking ^{125}I-IL-4 to IL-4R, Ohara et al. and Park et al. identified receptor proteins having apparent molecular weights of 60,000 and 75,000 daltons, respectively. It is possible that the small receptor size observed on the murine cells represents a proteolytically cleaved fragment of the native receptor. Subsequent experiments by Park et al. (*J.*

Exp. Med. 166:476, 1987) using yeast-derived recombinant human IL-4 radiolabeled with ^{125}I showed that human IL-4 receptor is present not only on cell lines of B, T, and hematopoietic cell lineages, but is also found on human fibroblasts and cells of epithelial and endothelial origin. IL-4 receptors have since been shown to be present on other cell lines, including CBA/N splenic B cells (Nakajima et al., *J. Immunol. 139*:774, 1987), Burkitt lymphoma Jijoye cells (Cabrillat et al., *Biochem. & Biophys. Res. Commun. 149*:995, 1987), a wide variety of hemopoietic and nonhemopoietic cells (Lowenthal et al., *J. Immunol. 140*:456, 1988), and murine Lyt-2$^-$/L3T4$^-$ thymocytes. More recently, Park et al. (UCLA Symposia, *J. Cell Biol.*, Suppl. 12A, 1988) reported that, in the presence of sufficient protease inhibitors, ^{125}I-IL-4-binding plasma membrane receptors of 138-145 kDa could be identified on several murine cell lines. Considerable controversy thus remains regarding the actual size and structure of IL-4 receptors.

Further study of the structure and biological characteristics of IL-4 receptors and the role played by IL-4 receptors in the responses of various cell populations to IL-4 or other cytokine stimulation, or of the methods of using IL-4 receptors effectively in therapy, diagnosis, or assay, has not been possible because of the difficulty in obtaining sufficient quantities of purified IL-4 receptor. No cell lines have previously been known to express high levels of IL-4 receptors constitutively and continuously, and in cell lines known to express detectable levels of IL-4 receptor, the level of expression is generally limited to less than about 2000 receptors per cell. Thus, efforts to purify the IL-4 receptor molecule for use in biochemical analysis or to clone and express mammalian genes encoding IL-4 receptor have been impeded by lack of purified receptor and a suitable source of receptor mRNA.

SUMMARY OF THE INVENTION

The present invention provides DNA sequences encoding mammalian interleukin-4 receptors (IL-4R) or subunits thereof. Preferably, such DNA sequences are selected from the group consisting of: (a) cDNA clones having a nucleotide sequence derived from the coding region of a native IL-4R gene; (b) DNA sequences capable of hybridization to the cDNA clones of (a) under moderately stringent conditions and which encode biologically active IL-4R molecules; and (c) DNA sequences which are degenerate, as a result of the genetic code, to the DNA sequences defined in (a) and (b) and which encode biologically active IL-4R molecules. The present invention also provides recombinant expression vectors comprising the DNA sequences defined above, recombinant IL-4R molecules produced using the recombinant expression vectors, and processes for producing the recombinant IL-4R molecules using the expression vectors.

The present invention also provides substantially homogeneous protein compositions comprising mammalian IL-4R. The full length murine molecule is a glycoprotein having a molecular weight of about 130,000 to about 140,000 M_r by SDS-PAGE. The apparent binding affinity (K_a) for COS cells transfected with murine IL-4 receptor clones 16 and 18 from the CTLL 19.4 library is 1 to 8 x 10^9 M^{-1}. The K_a for COS cells transfected with murine IL-4 receptor clones 7B9-2 and 7B9-4 from the murine 7B9 library is 2 x 10^9 to 1 x 10^{10} M^{-1}. The mature murine IL-4 receptor molecule has an N-terminal amino acid sequence as follows: IKVLGEPTCFSDYIRTSTCEW.

The human IL-4R molecule is believed to have a molecular weight of between about 110,000 and 150,000 M_r and has an N-terminal amino acid sequence, predicted from the cDNA sequence and by analogy to the biochemically determined N-terminal sequence of the mature murine protein, as follows: M K V L Q E P T C V S D Y M S I S T C E W.

The present invention also provides compositions for use in therapy, diagnosis, assay of IL-4 receptor, or in raising antibodies to IL-4 receptors, comprising effective quantities of soluble receptor proteins prepared according to the foregoing processes. Such soluble recombinant receptor molecules include truncated proteins wherein regions of the receptor molecule not required for IL-4 binding have been deleted. These and other aspects of the present invention will become evident upon reference to the following detailed description and attached drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows restriction maps of cDNA clones containing the coding regions (denoted by a bar) of the murine and human IL-4R cDNAs. The restriction sites *EcoR*I, *Pvu*II, *Hinc* II and *Sst* I are represented by the letters R, P, H and S, respectively.

Figures 2A-C depict the cDNA sequence and the derived amino acid sequence of the coding region of a murine IL-4 receptor, as derived from clone 7B9-2 of the 7B9 library. The N-terminal isoleucine of the mature protein is designated amino acid number 1. The coding region of the full-length membrane-bound protein from clone 7B9-2 is defined by amino acids 1-785. The ATC codon specifying the isoleucine residue constituting the mature N-terminus is underlined at position 1 of the protein sequence; the putative transmembrane region at amino acids 209-232 is also underlined. The sequences of the coding regions of clones 7B9-4 and clones CTLL-18 and CTLL-16 of the CTLL 19.4 library are identical to that of 7B9-2 except as follows. The coding region of CTLL-16 encodes a membrane-bound IL-4-binding receptor defined by amino acids -25 through 233 (including the putative 25 amino acid signal peptide sequence), but is followed by a TAG terminator codon (not shown) which ends the open reading frame. The nucleic acid sequence indicates the presence of a splice donor site at this position (indicated by an arrow in Figure 1) and a splice acceptor site near the 3' end (indicated by a second arrow), suggesting that CTLL-16 was derived from an unspliced mRNA intermediate. Clones 7B9-4 and CTLL-18 encode amino acids 23 through 199 and -25 through 199, respectively. After amino acid 199, a 114-base pair insert (identical in both clones and shown by an open box in Figure 1) introduces six new amino acids, followed by a termination codon. This form of the receptor is soluble.

Figure 3 is a schematic illustration of the mammalian high expression plasmid pCAV/NOT, which is described in greater detail in Example 8.

Figures 4A-C depict the coding sequence of a human IL-4 receptor cDNA from clone T22-8, which was obtained from a cDNA library derived from the T cell line T22. The predicted N-terminal methionine of the mature protein and the transmembrane region are underlined.

Figures 5A-B are a comparison of the predicted amino acid sequences of human (top line) and murine (bottom line) IL-4 receptor cDNA clones.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

As used herein, the terms "IL-4 receptor" and "IL-4R" refer to proteins having amino acid sequences which are substantially similar to the native mammalian interleukin-4 receptor amino acid sequences disclosed in Figures 2 and 4, and which are biologically active as defined below, in that they are capable of binding interleukin-4 (IL-4) molecules or transducing a biological signal initiated by an IL-4 molecule binding to a cell, or cross-reacting with anti-IL-4R antibodies raised against IL-4R from natural (i.e., nonrecombinant) sources. The native murine IL-4 receptor molecule is thought to have an apparent molecular weight by SDS-PAGE of about 140 kilodaltons (kDa). The terms "IL-4 receptor" or "IL-4R" include, but are not limited to, analogs or subunits of native proteins having at least 20 amino acids and which exhibit at least some biological activity in common with IL-4R. As used throughout the specification, the term "mature" means a protein expressed in a form lacking a leader sequence as may be present in full-length transcripts of a native gene. Various bioequivalent protein and amino acid analogs are described in detail below.

The term "substantially similar," when used to define either amino acid or nucleic acid sequences, means that a particular subject sequence, for example, a mutant sequence, varies from a reference sequence by one or more substitutions, deletions, or additions, the net effect of which is to retain biological activity of the IL-4R protein. Alternatively, nucleic acid subunits and analogs are "substantially similar" to the specific DNA sequences disclosed herein if: (a) the DNA sequence is derived from the coding region of a native mammalian IL-4R gene; (b) the DNA sequence is capable of hybridization to DNA sequences of (a) under moderately stringent conditions and which encode biologically active IL-4R molecules; or DNA sequences which are degenerate as a result of the genetic code to the DNA sequences defined in (a) or (b) and which encode biologically active IL-4R molecules. Substantially similar analog proteins will be greater than about 30 percent similar to the corresponding sequence of the native IL-4R. Sequences having lesser degrees of similarity but comparable biological activity are considered to be equivalents. More preferably, the analog proteins will be greater than about 80 percent similar to the corresponding sequence of the native IL-4R, in which case they are defined as being "substantially identical." In defining nucleic acid sequences, all subject nucleic acid sequences capable of encoding substantially similar amino acid sequences are considered substantially similar to a reference nucleic acid sequence. Percent similarity may be determined, for example, by comparing sequence information using the GAP computer program, version 6.0, available from the University of Wisconsin Genetics Computer Group (UWGCG). The GAP program utilizes the alignment method of Needleman and Wunsch (*J. Mol. Biol. 48*:443, 1970), as revised by Smith and Waterman (*Adv. Appl. Math. 2*:482, 1981). Briefly, the GAP program defines similarity as the number of aligned symbols (i.e., nucleotides or amino acids) which are similar, divided by the total number of symbols in the shorter of the two sequences. The preferred default parameters for the GAP program include: (1) a unary comparison matrix (containing a value of 1 for identities and 0 for non-identities) for nucleotides, and the weighted comparison matrix of Gribskov and Burgess,

Nucl. Acids Res. 14:6745, 1986, as described by Schwartz and Dayhoff, ed., *Atlas of Protein Sequence and Structure*, National Biomedical Research Foundation, pp. 353-358, 1979; (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps.

"Recombinant," as used herein, means that a protein is derived from recombinant (e.g., microbial or mammalian) expression systems. "Microbial" refers to recombinant proteins made in bacterial or fungal (e.g., yeast) expression systems. As a product, "recombinant microbial" defines a protein produced in a microbial expression system which is essentially free of native endogenous substances. Protein expressed in most bacterial cultures, e.g., *E. coli*, will be free of glycan. Protein expressed in yeast may have a glycosylation pattern different from that expressed in mammalian cells.

"Biologically active," as used throughout the specification as a characteristic of IL-4 receptors, means that a particular molecule shares sufficient amino acid sequence similarity with the embodiments of the present invention disclosed herein to be capable of binding detectable quantities of IL-4, transmitting an IL-4 stimulus to a cell, for example, as a component of a hybrid receptor construct, or cross-reacting with anti-IL-4R antibodies raised against IL-4R from natural (i.e., nonrecombinant) sources. Preferably, biologically active IL-4 receptors within the scope of the present invention are capable of binding greater than 0.1 nmoles IL-4 per nmole receptor, and most preferably, greater than 0.5 nmole IL-4 per nmole receptor in standard binding assays (see below).

"DNA sequence" refers to a DNA molecule, in the form of a separate fragment or as a component of a larger DNA construct, which has been derived from DNA isolated at least once in substantially pure form, i.e., free of contaminating endogenous materials and in a quantity or concentration enabling identification, manipulation, and recovery of the sequence and its component nucleotide sequences by standard biochemical methods, for example, using a cloning vector. Such sequences are preferably provided in the form of an open reading frame uninterrupted by internal nontranslated sequences, or introns, which are typically present in eukaryotic genes. Genomic DNA containing the relevant sequences could also be used. Sequences of non-translated DNA may be present 5' or 3' from the open reading frame, where the same do not interfere with manipulation or expression of the coding regions.

"Nucleotide sequence" refers to a heteropolymer of deoxyribonucleotides. DNA sequences encoding the proteins provided by this invention can be assembled from cDNA fragments and short oligonucleotide linkers, or from a series of oligonucleotides, to provide a synthetic gene which is capable of being expressed in a recombinant transcriptional unit.

"Recombinant expression vector" refers to a replicable DNA construct used either to amplify or to express DNA which encodes IL-4R and which includes a transcriptional unit comprising an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, promoters or enhancers, (2) a structural or coding sequence which is transcribed into mRNA and translated into protein, and (3) appropriate transcription and translation initiation and termination sequences. Structural elements intended for use in yeast expression systems preferably include a leader sequence enabling extracellular secretion of translated protein by a host cell. Alternatively,

where recombinant protein is expressed without a leader or transport sequence, it may include an N-terminal methionine residue. This residue may optionally be subsequently cleaved from the expressed recombinant protein to provide a final product.

"Recombinant microbial expression system" means a substantially homogeneous monoculture of suitable host microorganisms, for example, bacteria such as *E. coli* or yeast such as *S. cerevisiae*, which have stably integrated a recombinant transcriptional unit into chromosomal DNA or carry the recombinant transcriptional unit as a component of a resident plasmid. Generally, cells constituting the system are the progeny of a single ancestral transformant. Recombinant expression systems as defined herein will express heterologous protein upon induction of the regulatory elements linked to the DNA sequence or synthetic gene to be expressed.

Proteins and Analogs

The present invention provides substantially homogeneous recombinant mammalian IL-4R polypeptides substantially free of contaminating endogenous materials and, optionally, without associated native-pattern glycosylation. The native murine and human IL-4 receptor molecules are recovered from cell lysates as glycoproteins having an apparent molecular weight by SDS-PAGE of about 130-145 kilodaltons (kDa). Mammalian IL-4R of the present invention includes, by way of example, primate, human, murine, canine, feline, bovine, ovine, equine and porcine IL-4R. Derivatives of IL-4R within the scope of the invention also include various structural forms of the primary protein which retain biological activity. Due to the presence of ionizable amino and carboxyl groups, for example, an IL-4R protein may be in the form of acidic or basic salts, or in neutral form. Individual amino acid residues may also be modified by oxidation or reduction.

The primary amino acid structure may be modified by forming covalent or aggregative conjugates with other chemical moieties, such as glycosyl groups, lipids, phosphate, acetyl groups and the like, or by creating amino acid sequence mutants. Covalent derivatives are prepared by linking particular functional groups to IL-4R amino acid side chains or at the N- or C-termini. Other derivatives of IL-4R within the scope of this invention include covalent or aggregative conjugates of IL-4R or its fragments with other proteins or polypeptides, such as by synthesis in recombinant culture as N-terminal or C-terminal fusions. For example, the conjugated peptide may be a signal (or leader) polypeptide sequence at the N-terminal region of the protein which co-translationally or post-translationally directs transfer of the protein from its site of synthesis to its site of function inside or outside of the cell membrane or wall (e.g., the yeast α-factor leader). IL-4R protein fusions can comprise peptides added to facilitate purification or identification of IL-4R (e.g., poly-His). The amino acid sequence of IL-4 receptor can also be linked to the peptide Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys (DYKDDDDK) (Hopp et al., *Bio/Technology* 6:1204, 1988.) The latter sequence is highly antigenic and provides an epitope reversibly bound by a specific monoclonal antibody, enabling rapid assay and facile purification of expressed recombinant protein. This sequence is also specifically cleaved by bovine mucosal enterokinase at the residue immediately following the Asp-Lys pairing. Fusion proteins capped with this peptide may also be resistant to intracellular degradation in *E. coli*.

IL-4R derivatives may also be used as immunogens, reagents in receptor-based immunoassays, or as binding agents for affinity purification procedures of IL-4 or other binding ligands. IL-4R derivatives may also be obtained by cross-linking agents, such as M-maleimidobenzoyl succinimide ester and N-hydroxysuccinimide, at cysteine and lysine residues. IL-4R proteins may also be covalently bound through reactive side groups to various insoluble substrates, such as cyanogen bromide-activated, bisoxirane-activated, carbonyldiimidazole-activated or tosyl-activated agarose structures, or by adsorbing to polyolefin surfaces (with or without glutaraldehyde cross-linking). Once bound to a substrate, IL-4R may be used to selectively bind (for purposes of assay or purification) anti-IL-4R antibodies or IL-4.

The present invention also includes IL-4R with or without associated native-pattern glycosylation. IL-4R expressed in yeast or mammalian expression systems, e.g., COS-7 cells, may be similar or significantly different in molecular weight and glycosylation pattern than the native molecules, depending upon the expression system. Expression of IL-4R DNAs in bacteria such as *E. coli* provides non-glycosylated molecules. Functional mutant analogs of mammalian IL-4R having inactivated N-glycosylation sites can be produced by oligonucleotide synthesis and ligation or by site-specific mutagenesis techniques. These analog proteins can be produced in a homogeneous, reduced-carbohydrate form in good yield using yeast expression systems. N-glycosylation sites in eukaryotic proteins are characterized by the amino acid triplet Asn-A_1-Z, where A_1 is any amino acid except Pro, and Z is Ser or Thr. In this sequence, asparagine provides a side chain amino group for covalent attachment of carbohydrate. Such a site can be eliminated by substituting another amino acid for Asn or for residue Z, deleting Asn or Z, or inserting a non-Z amino acid between A_1 and Z, or an amino acid other than Asn between Asn and A_1.

IL-4R derivatives may also be obtained by mutations of IL-4R or its subunits. An IL-4R mutant, as referred to herein, is a polypeptide homologous to IL-4R but which has an amino acid sequence different from native IL-4R because of a deletion, insertion or substitution. Like most mammalian genes, mammalian IL-4 receptors are presumably encoded by multi-exon genes. Alternative mRNA constructs which can be attributed to different mRNA splicing events following transcription, and which share large regions of identity or similarity with the cDNAs claimed herein, are considered to be within the scope of the present invention.

Bioequivalent analogs of IL-4R proteins may be constructed by, for example, making various substitutions of residues or sequences or deleting terminal or internal residues or sequences not needed for biological activity. For example, cysteine residues can be deleted or replaced with other amino acids to prevent formation of incorrect intramolecular disulfide bridges upon renaturation. Other approaches to mutagenesis involve modification of adjacent dibasic amino acid residues to enhance expression in yeast systems in which KEX2 protease activity is present. Generally, substitutions should be made conservatively; i.e., the most preferred substitute amino acids are those having physicochemical characteristics resembling those of the residue to be replaced. Similarly, when a deletion or insertion strategy is adopted, the potential effect of the deletion or insertion on biological activity should be considered.

Subunits of IL-4R may be constructed by deleting terminal or internal residues or sequences. Particularly preferred subunits include those in which the transmembrane region and intracellular domain of IL-4R are deleted or substituted with hydrophilic residues to facilitate secretion of the receptor into the cell culture medium. The resulting protein is a soluble IL-4R molecule which may retain its ability to bind IL-4. Particular examples of soluble IL-4R include polypeptides having substantial identity to the sequence of amino acid residues 1-208 in Figure 2A, and residues 1-207 in Figure 4A.

Mutations in nucleotide sequences constructed for expression of analog IL-4Rs must, of course, preserve the reading frame phase of the coding sequences and preferably will not create complementary regions that could hybridize to produce secondary mRNA structures, such as loops or hairpins, which would adversely affect translation of the receptor mRNA. Although a mutation site may be predetermined, it is not necessary that the nature of the mutation *per se* be predetermined. For example, in order to select for optimum characteristics of mutants at a given site, random mutagenesis may be conducted at the target codon and the expressed IL-4R mutants screened for the desired activity.

Not all mutations in the nucleotide sequence which encodes IL-4R will be expressed in the final product, for example, nucleotide substitutions may be made to enhance expression, primarily to avoid secondary structure loops in the transcribed mRNA (see EPA 75,444A, incorporated herein by reference), or to provide codons that are more readily translated by the selected host, e.g., the well-known *E. coli* preference codons for *E. coli* expression.

Mutations can be introduced at particular loci by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes an analog having the desired amino acid insertion, substitution, or deletion.

Alternatively, oligonucleotide-directed site-specific mutagenesis procedures can be employed to provide an altered gene having particular codons altered according to the substitution, deletion, or insertion required. Exemplary methods of making the alterations set forth above are disclosed by Walder et al. (*Gene 42*:133, 1986); Bauer et al. (*Gene 37*:73, 1985); Craik (*BioTechniques*, January 1985, 12-19); Smith et al. (*Genetic Engineering: Principles and Methods*, Plenum Press, 1981); and U.S. Patent Nos. 4,518,584 and 4,737,462, which are incorporated by reference herein.

Expression of Recombinant IL-4R

The present invention provides recombinant expression vectors which include synthetic or cDNA-derived DNA fragments encoding mammalian IL-4R or bioequivalent analogs operably linked to suitable transcriptional or translational regulatory elements derived from mammalian, microbial, viral or insect genes. Such regulatory elements include a transcriptional promoter, an optional operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal binding sites, and sequences which control the termination of transcription and translation, as described in detail below.

The ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants may additionally be incorporated. DNA regions are operably linked when they are functionally related to each other. For example, DNA for a signal peptide (secretory leader) is operably linked to DNA for a polypeptide if it is expressed as a precursor which participates in the secretion of the polypeptide; a promoter is operably linked to a coding sequence if it controls the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to permit translation. Generally, operably linked means contiguous and, in the case of secretory leaders, contiguous and in reading frame.

DNA sequences encoding mammalian IL-4 receptors which are to be expressed in a microorganism will preferably contain no introns that could prematurely terminate transcription of DNA into mRNA; however, premature termination of transcription may be desirable, for example, where it would result in mutants having advantageous C-terminal truncations, for example, deletion of a transmembrane region to yield a soluble receptor not bound to the cell membrane. Due to code degeneracy, there can be considerable variation in nucleotide sequences encoding the same amino acid sequence; exemplary DNA embodiments are those corresponding to the nucleotide sequences shown in the Figures. Other embodiments include sequences capable of hybridizing to the sequences of the Figures under moderately stringent conditions (50°C, 2 X SSC) and other sequences hybridizing or degenerate to those described above, which encode biologically active IL-4 receptor polypeptides.

Transformed host cells are cells which have been transformed or transfected with IL-4R vectors constructed using recombinant DNA techniques. Transformed host cells ordinarily express IL-4R, but host cells transformed for purposes of cloning or amplifying IL-4R DNA do not need to express IL-4R. Expressed IL-4R will be deposited in the cell membrane or secreted into the culture supernatant, depending on the IL-4R DNA selected. Suitable host cells for expression of mammalian IL-4R include prokaryotes, yeast or higher eukaryotic cells under the control of appropriate promoters. Prokaryotes include gram negative or gram positive organisms, for example *E. coli* or bacilli. Higher eukaryotic cells include established cell lines of mammalian origin as described below. Cell-free translation systems could also be employed to produce mammalian IL-4R using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described by Pouwels et al. (*Cloning Vectors: A Laboratory Manual*, Elsevier, New York, 1985), the relevant disclosure of which is hereby incorporated by reference.

Prokaryotic expression hosts may be used for expression of IL-4Rs that do not require extensive proteolytic and disulfide processing. Prokaryotic expression vectors generally comprise one or more phenotypic selectable markers, for example a gene encoding proteins conferring antibiotic resistance or supplying an autotrophic requirement, and an origin of replication recognized by the host to ensure amplification within the host. Suitable prokaryotic hosts for transformation include *E. coli, Bacillus subtilis, Salmonella typhimurium*, and various species within the genera

Pseudomonas, *Streptomyces*, and *Staphylococcus*, although others may also be employed as a matter of choice.

Useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and pGEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed. *E. coli* is typically transformed using derivatives of pBR322, a plasmid derived from an *E. coli* species (Bolivar et al., *Gene* 2:95, 1977). pBR322 contains genes for ampicillin and tetracycline resistance and thus provides simple means for identifying transformed cells.

Promoters commonly used in recombinant microbial expression vectors include the β-lactamase (penicillinase) and lactose promoter system (Chang et al., *Nature 275*:615, 1978; and Goeddel et al., *Nature 281*:544, 1979), the tryptophan (trp) promoter system (Goeddel et al., *Nucl. Acids Res. 8*:4057, 1980; and EPA 36,776) and tac promoter (Maniatis, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, p. 412, 1982). A particularly useful bacterial expression system employs the phage λ P_L promoter and cI857ts thermolabile repressor. Plasmid vectors available from the American Type Culture Collection which incorporate derivatives of the λ P_L promoter include plasmid pHUB2, resident in *E. coli* strain JMB9 (ATCC 37092) and pPLc28, resident in *E. coli* RR1 (ATCC 53082).

Recombinant IL-4R proteins may also be expressed in yeast hosts, preferably from the *Saccharomyces* genus, such as *S. cerevisiae*. Yeast of other genera, such as *Pichia* or *Kluyveromyces* may also be employed. Yeast vectors will generally contain an origin of replication from the 2μ yeast plasmid or an autonomously replicating sequence (ARS), promoter, DNA encoding IL-4R, sequences for polyadenylation and transcription termination and a selection gene. Preferably, yeast vectors will include an origin of replication and selectable marker permitting transformation of both yeast and *E. coli*, e.g., the ampicillin resistance gene of *E. coli* and *S. cerevisiae* trp1 gene, which provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, and a promoter derived from a highly expressed yeast gene to induce transcription of a structural sequence downstream. The presence of the trp1 lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan.

Suitable promoter sequences in yeast vectors include the promoters for metallothionein, 3-phosphoglycerate kinase (Hitzeman et al., *J. Biol. Chem. 255*:2073, 1980) or other glycolytic enzymes (Hess et al., *J. Adv. Enzyme Reg. 7*:149, 1968; and Holland et al., *Biochem. 17*:4900, 1978), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. Suitable vectors and promoters for use in yeast expression are further described in Hitzeman, EPA 73,657.

Preferred yeast vectors can be assembled using DNA sequences from pBR322 for selection and replication in *E. coli* (Amp^r gene and origin of replication) and yeast DNA sequences including a

glucose-repressible ADH2 promoter and α-factor secretion leader. The ADH2 promoter has been described by Russell et al. (*J. Biol. Chem. 258*:2674, 1982) and Beier et al. (*Nature 300*:724, 1982). The yeast α-factor leader, which directs secretion of heterologous proteins, can be inserted between the promoter and the structural gene to be expressed. *See, e.g.,* Kurjan et al., *Cell 30*:933, 1982; and Bitter et al., *Proc. Natl. Acad. Sci. USA 81*:5330, 1984. The leader sequence may be modified to contain, near its 3' end, one or more useful restriction sites to facilitate fusion of the leader sequence to foreign genes.

Suitable yeast transformation protocols are known to those of skill in the art; an exemplary technique is described by Hinnen et al., *Proc. Natl. Acad. Sci. USA 75*:1929, 1978, selecting for Trp^+ transformants in a selective medium consisting of 0.67% yeast nitrogen base, 0.5% casamino acids, 2% glucose, 10 μg/ml adenine and 20 μg/ml uracil.

Host strains transformed by vectors comprising the ADH2 promoter may be grown for expression in a rich medium consisting of 1% yeast extract, 2% peptone, and 1% glucose supplemented with 80 μg/ml adenine and 80 μg/ml uracil. Derepression of the ADH2 promoter occurs upon exhaustion of medium glucose. Crude yeast supernatants are harvested by filtration and held at 4°C prior to further purification.

Various mammalian or insect cell culture systems can be employed to express recombinant protein. Baculovirus systems for production of heterologous proteins in insect cells are reviewed by Luckow and Summers, *Bio/Technology 6*:47 (1988). Examples of suitable mammalian host cell lines include the COS-7 lines of monkey kidney cells, described by Gluzman (*Cell 23*:175, 1981), and other cell lines capable of expressing an appropriate vector including, for example, L cells, C127, 3T3, Chinese hamster ovary (CHO), HeLa and BHK cell lines. Mammalian expression vectors may comprise nontranscribed elements such as an origin of replication, a suitable promoter and enhancer linked to the gene to be expressed, and other 5' or 3' flanking nontranscribed sequences, and 5' or 3' nontranslated sequences, such as necessary ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, and transcriptional termination sequences.

The transcriptional and translational control sequences in expression vectors to be used in transforming vertebrate cells may be provided by viral sources. For example, commonly used promoters and enhancers are derived from Polyoma, Adenovirus 2, Simian Virus 40 (SV40), and human cytomegalovirus. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early and late promoter, enhancer, splice, and polyadenylation sites may be used to provide the other genetic elements required for expression of a heterologous DNA sequence. The early and late promoters are particularly useful because both are obtained easily from the virus as a fragment which also contains the SV40 viral origin of replication (Fiers et al., *Nature 273*:113, 1978). Smaller or larger SV40 fragments may also be used, provided the approximately 250 bp sequence extending from the *Hind* III site toward the *Bgl* I site located in the viral origin of replication is included. Further, mammalian genomic IL-4R promoter, control and/or signal sequences may be utilized, provided such control sequences are compatible with the host cell chosen. Additional details regarding the use of a mammalian high expression vectors to produce a recombinant mammalian IL-4 receptor are provided

in Example 8 below. Exemplary vectors can be constructed as disclosed by Okayama and Berg (*Mol. Cell. Biol. 3*:280, 1983).

A useful system for stable high level expression of mammalian receptor cDNAs in C127 murine mammary epithelial cells can be constructed substantially as described by Cosman et al. (*Mol. Immunol. 23*:935, 1986).

A particularly preferred eukaryotic vector for expression of IL-4R DNA is disclosed below in Example 2. This vector, referred to as pCAV/NOT, was derived from the mammalian high expression vector pDC201 and contains regulatory sequences from SV40, adenovirus-2, and human cytomegalovirus. pCAV/NOT containing a human IL-7 receptor insert has been deposited with the American Type Culture Collection (ATCC) under deposit accession number 68014.

Purified mammalian IL-4 receptors or analogs are prepared by culturing suitable host/vector systems to express the recombinant translation products of the DNAs of the present invention, which are then purified from culture media or cell extracts.

For example, supernatants from systems which secrete recombinant protein into culture media can be first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a suitable purification matrix. For example, a suitable affinity matrix can comprise an IL-4 or lectin or antibody molecule bound to a suitable support. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. Sulfopropyl groups are preferred.

Finally, one or more reversed-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify an IL-4R composition. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a homogeneous recombinant protein.

Recombinant protein produced in bacterial culture is usually isolated by initial extraction from cell pellets, followed by one or more concentration, salting-out, aqueous ion exchange or size exclusion chromatography steps. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps. Microbial cells employed in expression of recombinant mammalian IL-4R can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

Fermentation of yeast which express mammalian IL-4R as a secreted protein greatly simplifies purification. Secreted recombinant protein resulting from a large-scale fermentation can be purified by methods analogous to those disclosed by Urdal et al. (*J. Chromatog. 296*:171, 1984). This reference describes two sequential, reversed-phase HPLC steps for purification of recombinant human IL-2 on a preparative HPLC column.

Human IL-4R synthesized in recombinant culture is characterized by the presence of non-human cell components, including proteins, in amounts and of a character which depend upon the purification steps taken to recover human IL-4R from the culture. These components ordinarily will be of yeast, prokaryotic or non-human higher eukaryotic origin and preferably are present in innocuous contaminant quantities, on the order of less than about 1 percent by weight. Further, recombinant cell culture enables the production of IL-4R free of proteins which may be normally associated with IL-4R as it is found in nature in its species of origin, e.g. in cells, cell exudates or body fluids.

IL-4R compositions are prepared for administration by mixing IL-4R having the desired degree of purity with physiologically acceptable carriers. Such carriers will be nontoxic to recipients at the dosages and concentrations employed. Ordinarily, the preparation of such compositions entails combining the IL-4R with buffers, antioxidants such as ascorbic acid, low molecular weight (less than about 10 residues) polypeptides, proteins, amino acids, carbohydrates including glucose, sucrose or dextrins, chelating agents such as EDTA, glutathione and other stabilizers and excipients.

IL-4R compositions may be used to regulate the function of B cells. For example, soluble IL-4R (sIL-4R) inhibits the proliferation of B cell cultures induced by IL-4 in the presence of anti-Ig. sIL-4R also inhibits IL-4 induced IgG1 secretion by LPS-activated B cells as determined by isotype specific ELISA and inhibits IL-4 induced Ia expression on murine B cells as determined by EPICS analysis. sIL-4R also inhibits IL-4 induced IgE synthesis and may accordingly be used to treat IgE-induced immediate hypersensitivity reactions, such as allergic rhinitis (common hay fever), bronchial asthma, atopic dermatitis and gastrointestinal food allergy.

IL-4R compositions may also be used to regulate the function of T cells. For example, IL-4R inhibits IL-4 induced proliferation of T cell lines, such as the CTLL T cell line. sIL-4R also inhibits functional activity mediated by endogenously produced IL-4. For example, sIL-4R inhibits the generation of alloreactive cytolytic T lymphocytes (CTL) in secondary mixed leukocyte culture when present in culture concomitantly with a monoclonal antibody against IL-2, such as S4B6. Neutralizing agents for both IL-2 and IL-4 are used to inhibit endogenous IL-2 and IL-4, both of which regulate CTL generation and are produced in such cultures.

In therapeutic applications, a therapeutically effective quantity of an IL-4 receptor composition is administered to a mammal, preferably a human, in association with a pharmaceutical carrier or diluent.

The following examples are offered by way of illustration, and not by way of limitation.

EXAMPLES

Example 1

Binding assays for IL-4 receptor

A. *Radiolabeling of IL-4.* Recombinant murine and human IL-4 were expressed in yeast and purified to homogeneity as described by Park, et al., *Proc. Natl. Acad. Sci. USA 84*:5267 (1987) and Park et al., *J. Exp. Med. 166*:476 (1987), respectively. The purified protein was radiolabeled using a commercially available enzymobead radioiodination reagent (BioRad). In this procedure 2.5 µg rIL-4 in

50 µl 0.2 M sodium phosphate, pH 7.2 are combined with 50 µl enzymobead reagent, 2 MCi of sodium iodide in 20 µl of 0.05 M sodium phosphate pH 7.0 and 10 µl of 2.5% b-D-glucose. After 10 min at 25°C, sodium azide (10 µl of 50 mM) and sodium metabisulfite (10 µl of 5 mg/ml) were added and incubation continued for 5 min. at 25°C. The reaction mixture was fractionated by gel filtration on a 2 ml bed volume of Sephadex® G-25 (Sigma) equilibrated in Roswell Park Memorial Institute (RPMI) 1640 medium containing 2.5% (w/v) bovine serum albumin (BSA), 0.2% (w/v) sodium azide and 20 mM Hepes pH 7.4 (binding medium). The final pool of ^{125}I-IL-4 was diluted to a working stock solution of 2 x 10^{-8} M in binding medium and stored for up to one month at 4°C without detectable loss of receptor binding activity. The specific activity is routinely in the range of 1-2 x 10^{16} cpm/mmole IL-4.

B. *Binding to Adherent Cells.* Binding assays done with cells grown in suspension culture (i.e., CTLL and CTLL-19.4) were performed by a phthalate oil separation method (Dower et al., *J. Immunol. 132*:751, 1984) essentially as described by Park et al., *J. Biol. Chem. 261*:4177, 1986 and Park et al., *supra.* Binding assays were also done on COS cells transfected with a mammalian expression vector containing cDNA encoding an IL-4 receptor molecule. For Scatchard analysis of binding to adherent cells, COS cells were transfected with plasmid DNA by the method of Luthman et al., *Nucl. Acids. Res. 11*:1295, 1983, and McCutchan et al., *J. Natl. Cancer Inst. 41*:351, 1968. Eight hours following transfection, cells were trypsinized, and reseeded in six well plates (Costar, Cambridge, MA) at a density of 1 x 10^4 COS-IL-4 receptor transfectants/well mixed with 5 x 10^5 COS control transfected cells as carriers. Two days later monolayers were assayed for ^{125}I-IL-4 binding at 4°C essentially by the method described by Park et al., *J. Exp. Med. 166*:476, 1987. Nonspecific binding of ^{125}I-IL-4 was measured in the presence of a 200-fold or greater molar excess of unlabeled IL-4. Sodium azide (0.2%) was included in all binding assays to inhibit internalization of ^{125}I-IL-4 by cells at 37°C.

For analysis of inhibition of binding by soluble IL-4R, supernatants from COS cells transfected with recombinant IL-4R constructs were harvested three days after transfection. Serial two-fold dilutions of conditioned media were pre-incubated with 3 x 10^{-10} M ^{125}I-IL-4 (having a specific activity of about 1 x 10^{16} cpm/mmol) for one hour at 37°C prior to the addition of 2 x 10^6 CTLL cells. Incubation was continued for 30 minutes at 37°C prior to separation of free and cell-bound murine ^{125}I-IL-4.

C. *Solid Phase Binding Assays.* The ability of IL-4 receptor to be stably adsorbed to nitrocellulose from detergent extracts of CTLL 19.4 cells yet retain IL-4 binding activity provided a means of monitoring purification. One ml aliquots of cell extracts (see Example 3), IL-4 affinity column fractions (see Example 4) or other samples are placed on dry BA85/21 nitrocellulose membranes (Schleicher and Schuell, Keene, NH) and allowed to dry. The membranes are incubated in tissue culture dishes for 30 minutes in Tris (0.05 M) buffered saline (0.15 M) pH 7.5 containing 3% w/v BSA to block nonspecific binding sites. The membrane is then covered with 4 x 10^{-11} M ^{125}I-IL-4 in PBS + 3% BSA with or without a 200 fold molar excess of unlabeled IL-4 and incubated for 2 hr at 4°C with shaking. At the end of this time, the membranes are washed 3 times in PBS, dried and placed on Kodak X-Omat™ AR film for 18 hr at -70°C.

Example 2

Selection of CTLL cells with high IL-4 receptor expression by fluorescence activated cell sorting (FACS)

The preferred cell line for obtaining high IL-4 receptor selection is CTLL, a murine IL-2 dependent cytotoxic T cell line (ATCC TIB 214). To obtain higher levels of IL-4 receptor expression, CTLL cells (parent cells) were sorted using fluorescence-activated cell sorting and fluorescein-conjugated recombinant murine IL-4 (rmIL-4) in which the extensive carbohydrate attached to rmIL-4 by the yeast host is used to advantage by coupling fluorescein hydrazide to periodate oxidized sugar moieties. The fluorescein-conjugated IL-4 was prepared by combining aliquots of hyperglycosylated rmIL-4 (300 µg in 300 µl of 0.1 M citrate-phosphate buffer, pH 5.5) with 30 µl of 10 mM sodium m-periodiate (Sigma), freshly prepared in 0.1 M citrate-phosphate, pH 5.5 and the mixture incubated at 4°C for 30 minutes in the dark. The reaction was quenched with 30 µl of 0.1 M glycerol and dialyzed for 18 hours at 4°C against 0.1 M citrate-phosphate pH 5.5. Following dialysis, a 1/10 volume of 100 mM 5-(((2-(carbohydrazino)methyl)thio)acetyl)-aminofluorescein (Molecular Probes, Eugene OR) dissolved in DMSO was added to the sample and incubated at 25°C for 30 minutes. The IL-4-fluorescein was then exhaustively dialyzed at 4°C against PBS, pH 7.4 and protein concentration determined by amino acid analysis. The final product was stored at 4°C following the addition of 1% (w/v) BSA and sterile filtration.

In order to sort, CTLL cells (5×10^6) were incubated for 30 min at 37°C in 150 µl PBS + 1% BSA containing 1×10^{-9} M IL-4-fluorescein under sterile conditions. The mixture was then chilled to 4°C, washed once in a large volume of PBS + 1% BSA and sorted using an EPICS® C flow cytometer (Coulter Instruments). The cells providing the highest level fluorescence signal (top 1.0%) were collected in bulk and the population expanded in liquid cell culture. Alternatively, for single cell cloning, cells exhibiting a fluorescence signal in the top 1.0% were sorted into 96 well tissue culture microtiter plates at 1 cell per well.

Progress was monitored by doing binding assays with ^{125}I-IL-4 following each round of FACS selection. Unsorted CTLL cells (CTLL parent) typically exhibited 1000-2000 IL-4 receptors per cell. CTLL cells were subjected to 19 rounds of FACS selection. The final CTLL cells selected (CTLL-19) exhibited 5×10^5 to 1×10^6 IL-4 receptors per cell. At this point the CTLL-19 population was subjected to EPICS® C-assisted single cell cloning and individual clonal populations were expanded and tested for ^{125}I-IL-4 binding. A single clone, designated CTLL-19.4, exhibited 1×10^6 IL-4 receptors per cell and was selected for purification and cloning studies. While the calculated apparent K_a values are similar for the two lines, CTLL-19.4 expresses approximately 400-fold more receptors on its surface than does the CTLL parent.

Example 3

Detergent extraction of CTLL cells

CTLL 19.4 cells were maintained in RPMI 1640 containing 10% fetal bovine serum, 50 U/ml penicillin, 50 µg/ml streptomycin and 10 ng/ml of recombinant human IL-2. Cells were grown to 5 x

10^5 cells/ml in roller bottles, harvested by centrifugation, washed twice in serum free DMEM and sedimented at 2000 x g for 10 minutes to form a packed pellet (about 2 x 10^8 cells/ml). To the pellet was added an equal volume of PBS containing 1% Triton® X-100 and a cocktail of protease inhibitors (2 mM phenylmethysulfonylfluoride, 10 µM pepstatin, 10 µM leupeptin, 2 mM o-phenanthroline and 2 mM EGTA). The cells were mixed with the extraction buffer by vigorous vortexing and the mixture incubated on ice for 20 minutes after which the mixture was centrifuged at 12,000 x g for 20 minutes at 8°C to remove nuclei and other debris. The supernatant was either used immediately or stored at -70°C until use.

Example 4

IL-4 receptor purification by IL-4 affinity chromatography

In order to obtain sufficient quantities of murine IL-4R to determine its N-terminal sequence or to further characterize human IL-4R, protein obtained from the detergent extraction of cells was further purified by affinity chromatography. Recombinant murine or human IL-4 was coupled to Affigel®-10 (BioRad) according to the manufacturer's suggestions. For example, to a solution of IL-4 (3.4 mg/ml in 0.4 ml of 0.1 M Hepes pH 7.4) was added 1.0 ml of washed Affigel®-10. The solution was rocked overnight at 4°C and an aliquot of the supernatant tested for protein by a BioRad protein assay per the manufacturer's instructions using BSA as a standard. Greater than 95% of the protein had coupled to the gel, suggesting that the column had a final load of 1.3 mg IL-4 per ml gel. Glycine ethyl ester was added to a final concentration of 0.05 M to block any unreacted sites on the gel. The gel was washed extensively with PBS-1% Triton® followed by 0.1 Glycine-HCl, pH 3.0. A 0.8 x 4.0 cm column was prepared with IL-4-coupled Affigel® prepared as described (4.0 ml bed volume) and washed with PBS containing 1% Triton® X-100 for purification of murine IL-4R. Alternatively, 50 µl aliquots of 20% suspension of IL-4-coupled Affigel® were incubated with ^{35}S-cysteine/methionine-labeled cell extracts for small-scale affinity purifications and gel electrophoresis.

Aliquots (25 ml) of detergent extracted IL-4 receptor bearing CTLL 19.4 cells were slowly applied to the murine IL-4 affinity column at 4°C (flow rate of 3.0 ml/hr). The column was then washed sequentially with PBS containing 1% Triton® X-100, RIPA buffer (0.05 M Tris, 0.15 M NaCl, 1% NP-40, 1% deoxycholate and 0.1% SDS), PBS containing 0.1% Triton® X-100 and 10 mM ATP, and PBS with 1% Triton® X-100 to remove all contaminating material except the mIL-4R. The column was then eluted with pH 3.0 glycine HCl buffer containing 0.1% Triton® X-100 to remove the IL-4R and washed subsequently with PBS containing 0.1% Triton® X-100. One ml fractions were collected for the elution and 2 ml fractions collected during the wash. Immediately following elution, samples were neutralized with 80 µl of 1 M Hepes, pH 7.4. The presence of receptor in the fractions was detected by the solid phase binding assay as described above, using ^{125}I-labeled IL-4. Aliquots were removed from each fraction for analysis by SDS-PAGE and the remainder frozen at -70°C until use. For SDS-PAGE, 40 µl of each column fraction was added to 40 µl of 2 X SDS sample buffer (0.125 M Tris HCl pH 6.8, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol). The samples were placed in a boiling water bath for 3 minutes and 80 µl aliquots applied to sample wells of a 10% polyacrylamide gel which was

set up and run according to the method of Laemmli (*Nature 227*:680, 1970). Following electrophoresis, gels were silver stained as previously described by Urdal et al. (*Proc. Natl. Acad. Sci. USA 81*:6481, 1984).

Purification by the foregoing process permitted identification by silver staining of polyacrylamide gels of two mIL-4R protein bands averaging 45 - 55 kDa and 30 - 40 kDa that were present in fractions exhibiting IL-4 binding activity. Experiments in which the cell surface proteins of CTLL-19.4 cells were radiolabeled and ^{125}I-labeled receptor was purified by affinity chromatography suggested that these two proteins were expressed on the cell surface. The ratio of the lower to higher molecular weight bands increased upon storage of fractions at 4°C, suggesting a precursor product relationship, possibly due to slow proteolytic degradation. The mIL-4 receptor protein purified by the foregoing process remains capable of binding IL-4, both in solution and when adsorbed to nitrocellulose.

Example 5

Sequencing of IL-4 receptor protein

CTLL 19.4 mIL-4 receptor containing fractions from the mIL-4 affinity column purification were prepared for amino terminal protein sequence analysis by fractionating on an SDS-PAGE gel and then transferred to a PVDF membrane. Prior to running the protein fractions on polyacrylamide gels, it was first necessary to remove residual detergent from the affinity purification process. Fractions containing proteins bound to the mIL-4 affinity column from three preparations were thawed and concentrated individually in a speed vac under vacuum to a final volume of 1 ml. The concentrated fractions were then adjusted to pH 2 by the addition of 50% (v/v) TFA and injected onto a Brownlees RP-300 reversed-phase HPLC column (2.1 x 30 mm) equilibrated with 0.1% (v/v) TFA in H_20 at a flow rate of 200 µl/min running on a Hewlett Packard Model 1090M HPLC. The column was washed with 0.1% TFA in H_20 for 20 minutes post injection. The HPLC column containing the bound protein was then developed with a gradient as follows:

Time	% Acetonitrile in 0.1% TFA
0	0
5	30
15	30
25	70
30	70
35	100
40	0

1 ml fractions were collected every five minutes and analyzed for the presence of protein by SDS PAGE followed by silver staining.

Each fraction from the HPLC run was evaporated to dryness in a speed vac and then resuspended in Laemmli reducing sample buffer, prepared as described by Laemmli, *U.K. Nature 227*:680, 1970. Samples were applied to a 5-20% gradient Laemmli SDS gel and run at 45 mA until the dye front reached the bottom of the gel. The gel was then transferred to PVDF paper and stained

as described by Matsudaira, *J. Biol. Chem. 262*:10035, 1987. Staining bands were clearly identified in fractions from each of the three preparations at approximately 30,000 to 40,000 M_r.

The bands from the previous PVDF blotting were excised and subjected to automated Edman degradation on an Applied Biosystems Model 477A Protein Sequencer essentially as described by March et al. (*Nature 315*:641, 1985), except that PTH amino acids were automatically injected and analyzed on line with an Applied Biosystems Model 120A HPLC using a gradient and detection system supplied by the manufacturer. The following amino terminal sequence was determined from the results of sequencing: NH_2-Ile-Lys-Val-Leu-Gly-Glu-Pro-Thr-Cys/Asn-Phe-Ser-Asp-Tyr-Ile. The bands from the second preparation used for amino terminal sequencing were treated with CNBr using the *in situ* technique described by March et al. (*Nature 315*: 641, 1985) to cleave the protein after internal methionine residues. Sequencing of the resulting cleavage products yielded the following data, indicating that the CNBr cleaved the protein after two internal methionine residues:

Cycle	Residues Observed
1	Val, Ser
2	Gly, Leu
3	Ile, Val
4	Tyr, Ser
5	Arg, Tyr
6	Glu, Thr
7	Asp, Ala
8	Asn, Leu
9	Pro, Val
10	Ala
11	Glu, Val
12	Phe, Gly
13	Ile, Asn
14	Val, Gln
15	Tyr, Ile
16	Lys, Asn
17	Val, Thr
18	Thr, Gly

When compared with the protein sequences derived from clones 16 and 18 (see Figure 2), the sequences matched as follows:

```
                1               5                  10                  15        18
Sequence 1: (Met)-Val-Asn-Ile-Ser-Arg-Glu-Asp-Asn-Pro-Ala-Glu-Phe-Ile-Val-Tyr-Asn-Val-Thr

                1               5                  10                  15        18
Sequence 2: (Met)-Ser-Gly-Val-Tyr-Tyr-Thr-Ala-Arg-Val-Arg-Val-Arg-Ser-Gln-Ile-Leu-Thr-Gly
```

Identical matches were found for all positions of sequence 1 except Asn(2) and sequence 2, except Arg at positions 8, 10, and 12, Ser at position 13, and Leu at position 16. The above sequences correspond to amino acid residues 137-154 and 169-187 of Figure 2.

In addition, the amino terminal sequence matched a sequence derived from the clone with position 9 being defined as a Cys.

The above data support the conclusion that clones 16 and 18 are derived from the message for the IL-4 receptor.

Example 6

Synthesis of hybrid-subtracted cDNA probe

In order to screen a library for clones encoding a murine IL-4 receptor, a highly enriched IL-4 receptor cDNA probe was obtained using a subtractive hybridization strategy. Polyadenylated (polyA+) mRNA was isolated from two similar cell lines, the parent cell line CTLL (which expresses approximately 2,000 receptors per cell) and the sorted cell line CTLL 19.4 (which expresses 1 x 10^6 receptors per cell). The mRNA content of these two cell lines is expected to be identical except for the relative level of IL-4 receptor mRNA. A radiolabeled single-stranded cDNA preparation was then made from the mRNA of the sorted cell line CTLL 19.4 by reverse transcription of polyadenylated mRNA from CTLL 19.4 cells by a procedure similar to that described by Maniatis et al., *Molecular Cloning, A Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1982). Briefly, polyA+ mRNA was purified as described by March et al. (*Nature 315*:641-647, 1985) and copied into cDNA by reverse transcriptase using oligo dT as a primer. To obtain a high level of ^{32}P-labeling of the cDNA,100 µCi of ^{32}P-dCTP (s.a.=3000 Ci/mmol) was used in a 50 µl reaction with non-radioactive dCTP at 10 µM. After reverse transcription at 42°C for 2 hours, EDTA was added to 20 mM and the RNA was hydrolyzed by adding NaOH to 0.2 M and incubating the cDNA mixture at 68°C for 20 minutes. The single-stranded cDNA was extracted with a phenol/chloroform (50/50) mixture previously equilibrated with 10 mM Tris-Cl, 1 mM EDTA. The aqueous phase was removed to a clean tube and made alkaline again by the addition of NaOH to 0.5 M. The cDNA was then size-fractionated by chromatography on a 6 ml Sephadex® G50 column in 30mM NaOH and 1 mM EDTA to remove small molecular weight contaminants.

The resulting size-fractionated cDNA generated from the sorted CTLL 19.4 cells was then hybridized with an excess of mRNA from the unsorted parental CTLL cells by ethanol-precipitating the cDNA from CTLL 19.4 cells with 30 µg of polyA+ mRNA isolated from unsorted CTLL cells, resuspending in 16 µl of 0.25 M $NaPO_4$, pH 6.8, 0.2% SDS, 2 mM EDTA and incubating for 20 hours at 68°C. The cDNAs from the sorted CTLL 19.4 cells that are complementary to mRNAs from the unsorted CTLL cells form double stranded cDNA/mRNA hybrids, which can then be separated from the single stranded cDNA based on their different binding affinities on hydroxyapatite. The mixture was diluted with 30 volumes of 0.02 M $NaPO_4$, pH 6.8, bound to hydroxyapatite at room temperature, and single-stranded cDNA was then eluted from the resin with 0.12 M $NaPO_4$, pH 6.8, at 60°C, as described by Sims et al., *Nature 312*:541, 1984. Phosphate buffer was then removed by centrifugation over 2 ml Sephadex® G50 spin columns in water. This hybrid subtraction procedure removes a majority of common sequences between CTLL 19.4 and unsorted CTLL cells, and leaves a single-stranded cDNA pool enriched for radiolabeled IL-4 receptor cDNA which can be used to probe a cDNA library (as described below).

Example 7

Synthesis of cDNA library and plaque screening

A cDNA library was constructed from polyadenylated mRNA isolated from CTLL 19.4 cells using standard techniques (Gubler, et al., *Gene 25*:263, 1983; Ausubel et al., eds., *Current Protocols in Molecular Biology*, Vol. 1, 1987). After reverse transcription using oligo dT as primer, the single-stranded cDNA was rendered double-stranded with DNA polymerase I, blunt-ended with T4 DNA polymerase, methylated with *EcoR* I methylase to protect *EcoR* I cleavage sites within the cDNA, and ligated to *EcoR* I linkers. The resulting constructs were digested with *EcoR* I to remove all but one copy of the linkers at each end of the cDNA, and ligated to an equimolar concentration of *EcoR* I cut and dephosphorylated λZAP® arms and the resulting ligation mix was packaged *in vitro* (Gigapack®) according to the manufacturer's instructions. Other suitable methods and reagents for generating cDNA libraries in λ phage vectors are described by Huynh et al., *DNA Cloning Techniques: A Practical Approach*, IRL Press, Oxford (1984); Meissner et al., *Proc. Natl. Acad. Sci. USA 84*:4171 (1987), and Ausubel et al., *supra*. λZAP® is a phage λ cloning vector similar to λgt11 (U.S. Patent 4,788,135) containing plasmid sequences from pUC19 (Norrander et al., *Gene 26*:101, 1987), a polylinker site located in a lacZ gene fragment, and an f1 phage origin of replication permitting recovery of ssDNA when host bacteria are superinfected with f1 helper phage. DNA is excised in the form of a plasmid comprising the foregoing elements, designated Bluescript®. Gigapack® is a sonicated *E. coli* extract used to package λ phage DNA. λZAP®, Bluescript®, and Gigapack® are registered trademarks of Stratagene, San Diego, CA, USA.

The radiolabeled hybrid-subtracted cDNA from Example 6 was then used as a probe to screen the cDNA library. The amplified library was plated on BB4 cells at a density of 25,000 plaques on each of 20 150 mm plates and incubated overnight at 37°C. All manipulations of λZAP® and excision of the Bluescript® plasmid were as described by Short et al., (*Nucl. Acids Res. 16*:7583, 1988) and Stratagene product literature. Duplicate plaque lift filters were incubated with hybrid-subtracted cDNA probes from Example 6 in hybridization buffer containing 50% formamide, 5 X SSC, 5 X Denhardt's reagent and 10% dextran sulfate at 42°C for 48 hours as described by Wahl et al., *Proc. Natl. Acad. Sci. USA76*:3683, 1979. Filters were then washed at 68°C in 0.2 X SSC. Sixteen positive plaques were purified for further analysis.

Bluescript® plasmids containing the cDNA inserts were excised from the phage as described by the manufacturer and transformed into *E. coli*. Plasmid DNA was isolated from individual colonies, digested with *EcoR* I to release the cDNA inserts and electrophoresed on standard 1% agarose gels. Four duplicate gels were blotted onto nylon filters to produce identical Southern blots for analysis with various probes which were (1) radiolabeled cDNA from unsorted CTLL cells, (2) radiolabeled cDNA from CTLL 19.4 sorted cells, (3) hybrid subtracted cDNA from CTLL 19.4 sorted cells, and (4) hybrid subtracted cDNA from CTLL 19.4 sorted cells after a second round of hybridization to poly A^+ mRNA from an IL-4 receptor negative mouse cell line (LBRM 33 1A5B6). These probes were increasingly enriched for cDNA copies of mRNA specific for the sorted cell line CTLL 19.4. Of the 16 positive

plaques isolated from the library, four clones (11A, 14, 16 and 18) showed a parallel increase in signal strength with enrichment of the probe.

Restriction mapping (shown in Figure 1) and DNA sequencing of the isolated CTLL clones indicated the existence of at least two distinct mRNA populations. Both mRNA types have homologous open reading frames over most of the coding region yet diverge at the 3' end, thus encoding homologous proteins with different COOH-terminal sequences. DNA sequence from inside the open reading frames of both clones code for protein sequence that is identical to protein sequence derived from sequencing of the purified IL-4 receptor described in more detail in Example 5. Clone 16 and clone 18 were used as the prototypes for these two distinct message types. Clone 16 contains an open reading frame that encodes a 258-amino acid polypeptide which includes amino acids -25 to 233 of Figure 2A. Clone 18 encodes a 230-amino acid polypeptide, the N-terminal 224 amino acids of which are identical to the N-terminus of clone 16 but diverge at the 3' end with nucleotides CCAAGTAATGAAAATCTG which encode the C-terminal 6 amino acids, Pro-Ser-Asn-Glu-Asn-Leu, followed by a termination codon TGA. Both clones were expressed in a mammalian expression system, as described in Example 8.

Example 8

Expression of IL-4R in mammalian cells

A. *Expression in COS-7 Cells.* A eukaryotic expression vector pCAV/NOT, shown in Figure 3, was derived from the mammalian high expression vector pDC201, described by Sims et al., *Science 241*:585, 1988). pDC201 is a derivative of pMLSV, previously described by Cosman et al., *Nature 312*:768, 1984. pCAV/NOT is designed to express cDNA sequences inserted at its multiple cloning site (MCS) when transfected into mammalian cells and includes the following components: SV40 (hatched box) contains SV40 sequences from coordinates 5171-270 including the origin of replication, enhancer sequences and early and late promoters. The fragment is oriented so that the direction of transcription from the early promoter is as shown by the arrow. CMV contains the promoter and enhancer regions from human cytomegalovirus (nucleotides -671 to +7 from the sequence published by Boshart et al., *Cell 41*:521-530, 1985). The tripartite leader (stippled box) contains the first exon and part of the intron between the first and second exons of the adenovirus-2 tripartite leader, the second exon and part of the third exon of the tripartite leader and a multiple cloning site (MCS) containing sites for *Xho* I, *Kpn* I, *Sma* I, *Not* I and *Bgl* II. pA (hatched box) contains SV40 sequences from 4127-4100 and 2770-2533 that include the polyadenylation and termination signals for early transcription. Clockwise from pA are adenovirus-2 sequences 10532-11156 containing the VAI and VAII genes (designated by a black bar), followed by pBR322 sequences (solid line) from 4363-2486 and 1094-375 containing the ampicillin resistance gene and origin of replication. The resulting expression vector was designated pCAV/NOT.

Inserts in clone 16 and clone 18 were both released from Bluescript® plasmid by digestion with *Asp* 718 and *Not* I. The 3.5 kb insert from clone 16 was then ligated directly into the expression vector pCAV/NOT also cut at the *Asp* 718 and *Not* I sites in the polylinker region. The insert from

clone 18 was blunt-ended with T4 polymerase followed by ligation into the vector pCAV/NOT cut with *Sma* I and dephosphorylated.

Plasmid DNA from both IL-4 receptor expression plasmids were used to transfect a sub-confluent layer of monkey COS-7 cells using DEAE-dextran followed by chloroquine treatment, as described by Luthman et al. (*Nucl. Acids Res. 11*:1295, 1983) and McCutchan et al. (*J. Natl. Cancer Inst. 41*:351, 1968). The cells were then grown in culture for three days to permit transient expression of the inserted sequences. After three days, cell culture supernatants and the cell monolayers were assayed (as described in Example 1) and IL-4 binding was confirmed.

B. *Expression in CHO Cells.* IL-4R was also expressed in the mammalian CHO cell line by first ligating an *Asp*718/*Not*I restriction fragment of clone 18 into the pCAV/NOT vector as described in Example 8. The pCAV/NOT vector containing the insert from clone 18 was then co-transfected using a standard calcium phosphate method into CHO cells with the dihydrofolate reductase (DHFR) cDNA selectable marker under the control of the SV40 early promoter. The DHFR sequence enables methotrexate selection for mammalian cells harboring the plasmid. DHFR sequence amplification events in such cells were selected using elevated methotrexate concentrations. In this way, the contiguous DNA sequences are also amplified and thus enhanced expression is achieved. Mass cell cultures of the transfectants secreted active soluble IL-4R at approximately 100 ng/ml.

C. *Expression in HeLa Cells.* IL-4R was expressed in the human HeLa-EBNA cell line 653-6, which constitutively expresses EBV nuclear antigen-1 driven from the CMV immediate-early enhancer/promoter. The expression vector used was pHAV-EO-NEO, described by Dower et al., *J. Immunol. 142*:4314, 1989), a derivative of pDC201, which contains the EBV origin of replication and allows high level expression in the 653-6 cell line. pHAV-EO-NEO is derived from pDC201 by replacing the adenovirus major late promoter with synthetic sequences from HIV-1 extending from -148 to +78 relative to the cap site of the viral mRNA, and including the HIV-1 *tat* gene under the control of the SV-40 early promoter. It also contains a *Bgl* II-*Sma* I fragment containing the neomycin resistance gene of pSV2NEO (Southern & Berg, *J. Mol. Appl. Genet. 1*:332, 1982) inserted into the *Bgl* II and *Hpa* I sites and subcloning downstream of the *Sal* I cloning site. The resulting vector permits selection of transfected cells for neomycin resistance.

A 760 bp IL-4R fragment was released form the Bluescript® plasmid by digesting with *EcoN* I and *Sst* I restriction enzymes. This fragment of clone 18 corresponds to nucleotides 1-672 of Figure 2A, with the addition of a 5' terminal nucleotide sequence of GTGCAGGCACCTTTTGTGTCCCCA, a TGA stop codon which follows nucleotide 672 of Figure 2A, and a 3' terminal nucleotide sequence of CTGAGTGACCTTGGGGGCTGCGGTGGTGAGGAGAGCT. This fragment was then blunt-ended using T4 polymerase and subcloned into the *Sal* I site of pHAV-EO-NEO. The resulting plasmid was then transfected into the 653-6 cell line by a modified polybrene transfection method as described by Dower et al. (*J. Immunol. 142*:4314, 1989) with the exception that the cells were trypsinized at 2 days post-transfection and split at a ratio of 1:8 into media containing G418 (Gibco Co.) at a concentration of 1 mg/ml. Culture media were changed twice weekly until neomycin-resistant colonies were established. Colonies were then either picked individually using cloning rings, or pooled together, to

generate several different cell lines. These cell lines were maintained under drug selection at a G418 concentration of 250 µg/ml. When the cells reached confluency supernatants were taken and tested in the inhibition assay of Example 1B. Cell lines produced from 100 ng/ml to 600 ng/ml of soluble IL-4R protein.

Example 9

Expression of IL-4R in yeast cells

For expression of mIL-4R, a yeast expression vector derived from pIXY120 was constructed as follows. pIXY120 is identical to pYαHuGM (ATCC 53157), except that it contains no cDNA insert and includes a polylinker/multiple cloning site with an *Nco* I site. This vector includes DNA sequences from the following sources: (1) a large *Sph* I (nucleotide 562) to *EcoR* I (nucleotide 4361) fragment excised from plasmid pBR322 (ATCC 37017), including the origin of replication and the ampicillin resistance marker for selection in *E. coli*; (2) *S. cerevisiae* DNA including the TRP-1 marker, 2µ origin of replication, ADH2 promoter; and (3) DNA encoding an 85 amino acid signal peptide derived from the gene encoding the secreted peptide α-factor (See Kurjan et al., U.S. Patent 4,546,082). An *Asp* 718 restriction site was introduced at position 237 in the α-factor signal peptide to facilitate fusion to heterologous genes. This was achieved by changing the thymidine residue at nucleotide 241 to a cytosine residue by oligonucleotide-directed *in vitro* mutagenesis as described by Craik, *BioTechniques*, January 1985, pp.12-19. A synthetic oligonucleotide containing multiple cloning sites and having the following sequence was inserted from the *Asp*718 site at amino acid 79 near the 3' end of the α-factor signal peptide to a *Spe*I site in the 2µ sequence:

```
Asp718                                  StuI    NcoI        BamHI SmaI      SpeI
GTACCTTTGGATAAAAGAGACTACAAGGACGACGATGACAAGAGGCCTCCATGGATCCCCCGGGACA
   GAAACCTATTTTCTCTGATGTTCCTGCTGCTACTGTTCTCCGGAGGTACCTAGGGGGCCCTGTGATC
                                          |<----------Polylinker----------->|
```

pBC120 also varies from pYαHuGM by the presence of a 514 bp DNA fragment derived from the single-stranded phage f1 containing the origin of replication and intergenic region, which has been inserted at the *Nru* I site in the pBR322 sequence. The presence of an f1 origin of replication permits generation of single-stranded DNA copies of the vector when transformed into appropriate strains of *E. coli* and superinfected with bacteriophage f1, which facilitates DNA sequencing of the vector and provides a basis for *in vitro* mutagenesis. To insert a cDNA, pIXY120 is digested with *Asp* 718 which cleaves near the 3' end of the α-factor leader peptide (nucleotide 237) and, for example, *BamH* I which cleaves in the polylinker. The large vector fragment is then purified and ligated to a DNA fragment encoding the protein to be expressed.

To create a secretion vector for expressing mIL-4R, a cDNA fragment encoding mIL-4R was excised from the Bluescript® plasmid of Example 8 by digestion with *Ppum* I and *Bgl* II to release an 831 bp fragment from the *Ppum* I site (see FIGURE) to an *Bgl* II site located 3' to the open reading frame containing the mIL-4R sequence minus the first two 5' codons encoding Ile and Lys. pIXY120 was digested with *Asp* 718 near the 3' end of the α-factor leader and *BamH* I. The vector fragment was

ligated to the *Ppum I/Bgl* II hIL-4R cDNA fragment and the following fragment created by annealing a pair of synthetic oligonucleotides to recreate the last 6 amino acids of the α-factor leader and the first two amino acids of mature mIL-4R.

```
                α-factor processing —>|
GTA CCT CTA GAT AAA AGA ATC AAG
     GA GAT CTA TTT TCT TAG TTC CAG
Val Pro Leu Asp Lys Arg Ile Lys
                                |<— mIL-4R
```

The oligonucleotide also included a change from the nucleotide sequence TGG ATA to CTA GAT which introduces a *Xba* I restriction site, without altering the encoded amino acid sequence.

The foregoing expression vector was then purified and employed to transform a diploid yeast strain of *S. cerevisiae* (XV2181) by standard techniques, such as those disclosed in EPA 165,654, selecting for tryptophan prototrophs. The resulting transformants were cultured for expression of a secreted mIL-4R protein. Cultures to be assayed for biological activity were grown in 20-50 ml of YPD medium (1% yeast extract, 2% peptone, 1% glucose) at 37°C to a cell density of 1-5 x 10^8 cells/ml. To separate cells from medium, cells were removed by centrifugation and the medium filtered through a 0.45 μ cellulose acetate filter prior to assay. Supernatants produced by the transformed yeast strain, or crude extracts prepared from disrupted yeast cells transformed the plasmid, were assayed to verify expression of a biologically active protein.

Example 10

Isolation of full-length and truncated forms of murine IL-4 receptor cDNAs from unsorted 7B9 cells

Polyadenylated RNA was isolated from 7B9 cells, an antigen-dependent helper T cell clone derived from C57BL/6 mice, and used to construct a cDNA library in λZAP (Stratagene, San Diego), as described in example 7. The λZAP library was amplified once and a total of 300,000 plaques were screened as described in Example 7, with the exception that the probe was a randomly primed ^{32}P-labeled 700 bp *EcoR* I fragment isolated from CTLL 19.4 clone 16. Thirteen clones were isolated and characterized by restriction analysis.

Nucleic acid sequence analysis of clone 7B9-2 revealed that it contains a polyadenylated tail, a putative polyadenylation signal, and an open reading frame of 810 amino acids (shown in Fig. 2), the first 258 of which are identical to those encoded by CTLL 19.4 clone 16, including the 25 amino acid putative signal peptide sequence. The 7B9-2 cDNA was subcloned into the eukaryotic expression vector, pCAV/NOT, and the resulting plasmid was transfected into COS-7 cells as described in Example 8. COS-7 transfectants were analyzed as set forth in Example 12.

A second cDNA form, similar to clone 18 in the CTLL 19.4 library, was isolated from the 7B9 library and subjected to sequence analysis. This cDNA, clone 7B9-4, is 376 bp shorter than clone 7B9-2 at the 5' end, and lacks the first 47 amino acids encoded by 7B9-2, but encodes the remaining N-terminal amino acids 23-199 (in Fig. 2). At position 200, clone 7B9-4 (like clone 18 from CTLL 19.4) has a 114 bp insert which changes the amino acid sequence to Pro Ser Asn Glu Asn Leu followed by a

termination codon. The 114 bp inserts, found in both clone 7B9-4 and CTLL 19.4 clone 18 are identical in nucleic acid sequence. The fact that this cDNA form, which produces a secreted form of the IL-4 receptor when expressed in COS-7 cells, was isolated from these two different cell lines indicates that it is neither a cloning artifact nor a mutant form peculiar to the sorted CTLL cells.

Example 11

Isolation of human IL-4 receptor cDNAs from PBL and T22 libraries by cross-species hybridization

Polyadenylated RNA was isolated from pooled human peripheral blood lymphocytes (PBL) that were obtained by standard Ficoll purification and were cultured in IL-2 for six days followed by stimulation with PMA and Con-A for eight hours. An oligo dT primed cDNA library was constructed in λgt10 using techniques described in example 7. A probe was produced by synthesizing an unlabeled RNA transcript of the 7B9-4 cDNA insert using T7 RNA polymerase, followed by ^{32}P-labeled cDNA synthesis with reverse transcriptase using random primers (Boehringer-Mannheim). This murine single-stranded cDNA probe was used to screen 50,000 plaques from the human cDNA library in 50% formamide/0.4 M NaCl at 42°C, followed by washing in 2 X SSC at 55°C. Three positive plaques were purified, and the *EcoR* I inserts subcloned into the Bluescript® plasmid vector. Nucleic acid sequencing of a portion of clone PBL-1, a 3.4 kb cDNA, indicated the clone was approximately 67% homologous to the corresponding sequence of the murine IL-4 receptor. However, an insert of 68 bp, containing a termination codon and bearing no homology to the mouse IL-4 receptor clones, was found 45 amino acids downstream of the predicted N-terminus of the mature protein, suggesting that clone PBL-1 encodes a non-functional truncated form of the receptor. Nine additional human PBL clones were obtained by screening the same library (under stringent conditions) with a ^{32}P-labeled random-primed probe made from the clone PBL-1 (the 3.4 kb *EcoR* I cDNA insert). Two of these clones, PBL-11 and PBL-5, span the 5' region that contains the 68 bp insert in PBL-1, but lack the 68 bp insert and do not extend fully 3', as evidenced by their size, thus precluding functional analysis by mammalian expression. In order to obtain a construct expressible in COS-7 cells, the 5' *Not* I-*Hinc* II fragment of clones PBL-11 and PBL-5 were separately ligated to the 3' *Hinc* II-*BamH* I end of clone PBL-1, and subcloned into the pCAV/NOT expression vector cut with *Not* I and *Bgl* II described in Example 8. These chimeric human IL-4R cDNAs containing PBL-11/PBL-1 and PBL-5/PBL-1 DNA sequences have been termed clones A5 and B4, respectively, as further described in Example 12. These constructs were transfected into COS-7 cells, and assayed for IL-4 binding in a plate binding assay substantially as described in Sims et al. (*Science 241*:585, 1988). Both composite constructs encoded protein which exhibited IL-4 binding activity. The nucleotide sequence and predicted amino acid sequence of the composite A5 construct correspond to the sequence information set forth in Figures 4A-4C, with the exception that a GTC codon encodes the amino acid Val at position 50, instead of Ile. No other clones that were sequenced contained this change. The consensus codon from clones PBL-1, PBL-5 and T22-8, however, is ATC and encodes Ile^{50}, as set forth in Figure 4A. The nucleotide and predicted amino acid sequence of the composite B4 construct also shows that

the 25 amino acid leader sequence of PBL-11 is replaced with the sequence Met-Gln-Lys-Asp-Ala-Arg-Arg-Glu-Gly-Asn.

Constructs expressing a soluble form of the human IL-4 receptor were made by excising a 5'-terminal 0.8 kb *Sma* I-*Dra* III fragment from PBL-5 and the corresponding 0.8 kb Asp718-*Dra* III fragment from PBL-11, of which the *Dra* III overhangs were blunt-ended with T4 polymerase. The PBL-5 and PBL-11 fragments were separately subcloned into CAV/NOT cut with *Sma* I or *Asp* 718 plus *Sma* I, respectively; these are called soluble hIL-4R-5 and soluble hIL-4R-11, respectively.

A second library made from a CD4+/CD8- human T cell clone, T22, (Acres et al., *J. Immunol. 138*:2132, 1987) was screened (using duplicate filters) with two different probes synthesized as described above. The first probe was obtained from a 220 bp *Pvu* II fragment from the 5' end of clone PBL-1 and the second probe was obtained from a 300 bp *Pvu* II -*EcoR* I fragment from the 3' end of clone PBL-1. Five additional cDNA clones were identified using these two probes. Two of these clones span the 5' region containing the 68 bp insert, but neither contain the insert. The third of these clones, T22-8, was approximately 3.6 kb in size and contained an open reading frame of 825 amino acids, including a 25 amino acid leader sequence, a 207 amino acid mature external domain, a 24 amino acid transmembrane region and a 569 amino acid cytoplasmic domain. The sequence of clone T22-8 is set forth in Figures 4A-4C. Figures 5A-5B compare the predicted human IL-4R amino acid sequence with the predicted murine IL-4R sequence and show approximately 53% sequence identity between the two proteins.

Example 12

Analysis and purification of IL-4 receptor in COS transfectants

Equilibrium binding studies were conducted for COS cells transfected with murine IL-4 receptor clones 16 and 18 from the CTLL 19.4 library. In all cases analysis of the data in the Scatchard coordinate system (Scatchard, *Ann. N.Y. Acad. Sci. 51*:660-672, 1949) yielded a straight line, indicating a single class of high-affinity receptors for murine IL-4. For COS pCAV-16 cells the calculated apparent K_a was 3.6×10^9 M^{-1} with 5.9×10^5 specific binding sites per cell. A similar apparent K_a was calculated for COS pCAV-18 cells at 1.5×10^9 M^{-1} but receptor number expressed at the cell surface was 4.2×10^4. Equilibrium binding studies performed on COS cells transfected with IL-4R DNA clones isolated from the 7B9 cell library also showed high affinity binding of the receptor to IL-4. Specifically, studies using COS cells transfected with pCAV-7B9-2 demonstrated that the full length murine IL-4 receptor bound ^{125}I-IL-4 with an apparent K_a of about 1.4×10^{10} M^{-1} with 4.5×10^4 specific binding sites per cell. The apparent K_a of CAV-7B9-4 IL-4R was calculated to be about 1.7×10^9 M^{-1}. Although absolute values for K_a and binding sites per cell varied between transfections, the binding affinities were generally similar (1×10^9 - 1×10^{10} M^{-1}) and matched well with previously published affinity constants for IL-4 binding.

Inhibition of ^{125}I-mIL-4 binding to CTLL cells by conditioned media from COS cells transfected with plasmid pCAV, pCAV-18, or pCAV-7B9-4 was used to determine if these cDNAs encoded functional soluble receptor molecules. Approximately 1.5 µl of COS pCAV-18 conditioned media in a

final assay volume of 150 µl gives approximately 50% inhibition of ^{125}I-IL-4 binding to the IL-4 receptor on CTLL cells. ^{125}I-IL-4 receptor competing activity is not detected in control pCAV transfected COS supernatants. From quantitative analysis of the dilution of pCAV-18 supernatant required to inhibit ^{125}I-IL-4 binding by 50%, it is estimated that approximately 60-100 ng/ml of soluble IL-4 receptor has been secreted by COS cells when harvested three days after transfection. Similar results were obtained utilizing supernatants from COS cells transfected with pCAV-7B9-4.

Conditioned medium from COS cells transfected with pCAV-18 or pCAV-7B9-4 (see Example 8) and grown in DMEM containing 3% FBS was harvested three days after transfection. Supernatants were centrifuged at 3,000 cpm for 10 minutes, and frozen until needed. Two hundred ml of conditioned media was loaded onto a column containing 4 ml of muIL-4 Affigel prepared as described above. The column was washed extensively with PBS and IL-4 receptor eluted with 0.1 M glycine, 0.15 M NaCl pH 3.0. Immediately following elution, samples were neutralized with 80 µl of 1 M Hepes pH 7.4. Samples were tested for their ability to inhibit binding of ^{125}I-muIL-4 to CTLL cells as set forth in Example 1B. Additionally samples were tested for purity by analysis on SDS-PAGE and silver staining as previously described. Alternative methods for testing functional soluble receptor activity or IL-4 binding inhibition include solid-phase binding assays, as described in Example 1C, or other similar cell free assays which may utilize either radio iodinated or colorimetrically developed IL-4 binding, such as RIA or ELISA. The protein analyzed by SDS-PAGE under reducing conditions has a molecular weight of approximately 37,500, and appears approximately 90% pure by silver stain analysis of gels.

Purified recombinant soluble murine IL-4 receptor protein may also be tested for its ability to inhibit IL-4 induced ^{3}H-thymidine incorporation in CTLL cells. Pursuant to such methods, soluble IL-4 receptor has been found to block IL-4 stimulated proliferation, but does not affect IL-2 driven mitogenic response.

Molecular weight estimates were performed on mIL-4 receptor clones transfected into COS cells. Utilizing M2 monoclonal antibody prepared against murine CTLL 19.4 cells (see Example 13), IL-4 receptor is immunoprecipitated from COS cells transfected with CAV-16, CAV-7B9-2 and CAV-7B9-4 and labeled with ^{35}S-cysteine and ^{35}S-methionine. Cell associated receptor from CAV-7B9-4 shows molecular weight heterogeneity ranging from 32-39 kDa. Secreted CAV-7B9-4 receptor has molecular weight between 36 and 41 kDa. Cell associated receptor from CAV-16 transfected COS cells is about 40-41 kDa. This is significantly smaller than molecular weight estimations from crosslinking studies described by Park et al., *J. Exp. Med. 166*:476, 1987; *J. Cell. Biol.*, Suppl. 12A, 1988. Immunoprecipitation of COS CAV-7B9-2 cell-associated receptor showed a molecular weight of 130-140 kDa, similar to the estimates of Park et al., *J. Cell. Biol.*, Suppl. 12A, 1988, estimated to be the full length IL-4 receptor. Similar molecular weight estimates of cell-associated CAV-16 and CAV-7B9-2 IL-4 receptor have also been made based on cross-linking ^{125}IL-4 to COS cells transfected with these cDNAs. Heterogeneity of molecular weight of the individual clones can be partially attributed to glycosylation. This data, together with DNA sequence analysis, suggests that the 7B9-2 cDNA encodes the full length cell-surface IL-4 receptor, whereas both 7B9-4 and clone 18 represent soluble forms of murine IL-4 receptor.

Receptor characterization studies were also done on COS cells transfected with hIL-4R containing expression plasmids. The two chimeric human IL-4R molecules A5 and B4 (defined in Example 11) were transfected into COS cells and equilibrium binding studies undertaken. The COS monkey cell itself has receptors capable of binding hIL-4; therefore the binding calculations performed on COS cells transfected with and overexpressing hIL-4R cDNAs represent background binding from endogenous monkey IL-4R molecules subtracted from the total binding. COS cells transfected with hIL-4R A5 had 5.3×10^4 hIL-4 binding sites with a calculated K_a of 3.48×10^9 M^{-1}. Similarly, the hIL-4R B4 expressed in COS cells bound ^{125}I-hIL-4 with an affinity of 3.94×10^9 M^{-1} exhibiting 3.2×10^4 receptors per cell.

Molecular weight estimates of human IL-4R expressed in COS cells were also performed. COS cells transfected with clones A5 or B4 in pCAV/NOT were labeled with ^{35}S-cysteine/ methionine and lysed. Human IL-4R was affinity purified from the resulting lysates with hIL-4-coupled Affigel® (as described in Example 4). The hIL-4R A5 and B4 eluted from this affinity support migrated at about 140,000 daltons on SDS-PAGE, agreeing well with previous estimates of hIL-4R molecular weight by cross-linking (Park et al., *J. Exp. Med. 166*:476, 1987), as well as with estimates of full-length mIL-4R presented here.

Because no soluble human IL-4R cDNA has thus far been found occurring naturally, as was the case for the murine receptor (clones 18 and 7B9-4), a truncated form was constructed as described in Example 11. Following expression in COS cells, supernatants were harvested three days after transfection with soluble hIL-4R-11 and soluble hIL-4R-5 and tested for inhibition of ^{125}I-hIL-4 binding to the human B cell line Raji. Supernatants from two of the soluble hIL-4R-11 and one of the soluble hIL-4R-5 transfected plates contained 29-149 ng/ml of IL-4R competing activity into the medium. In addition, the truncated protein could be detected in ^{35}S-methionine/cysteine-labeled COS cell transfectants by affinity purification on hIL-4-coupled Affigel® as approximately a 44 kDa protein by SDS-PAGE.

Example 13

Preparation of monoclonal antibodies to IL-4R

Preparations of purified recombinant IL-4 receptor, for example, human or murine IL-4 receptor, transfected COS cells expressing high levels of IL-4 receptor or CTLL 19.4 cells are employed to generate monoclonal antibodies against IL-4 receptor using conventional techniques, such as those disclosed in U. S. Patent 4,411,993. Such antibodies are likely to be useful in interfering with IL-4 binding to IL-4 receptors, for example, in ameliorating toxic or other undesired effects of IL-4.

To immunize rats, IL-4 receptor bearing CTLL 19.4 cells were used as immunogen emulsified in complete Freund's adjuvant and injected in amounts ranging from 10-100 µl subcutaneously into Lewis rats. Three weeks later, the immunized animals were boosted with additional immunogen emulsified in incomplete Freund's adjuvant and boosted every three weeks thereafter. Serum samples are periodically taken by retro-orbital bleeding or tail-tip excision for testing by dot-blot assay,

ELISA (enzyme-linked immunosorbent assay), or inhibition of binding of ^{125}I-IL-4 to extracts of CTLL cells (as described in Example 1). Other assay procedures are also suitable. Following detection of an appropriate antibody titer, positive animals were given a final intravenous injection of antigen in saline. Three to four days later, the animals were sacrificed, splenocytes harvested, and fused to the murine myeloma cell line AG8653. Hybridoma cell lines generated by this procedure were plated in multiple microtiter plates in a HAT selective medium (hypoxanthine, aminopterin, and thymidine) to inhibit proliferation of non-fused cells, myeloma hybrids, and spleen cell hybrids.

Hybridoma clones thus generated were screened for reactivity with IL-4 receptor. Initial screening of hybridoma supernatants utilized an antibody capture and binding of partially purified ^{125}I-mIL-4 receptor. Two of over 400 hybridomas screened were positive by this method. These two monoclonal antibodies, M1 and M2, were tested by a modified antibody capture to detect blocking antibody. Only M1 was able to inhibit ^{125}I-rmIL-4 binding to intact CTLL cells. Both antibodies are capable of immunoprecipitating native mIL-4R protein from CTLL cells or COS-7 cells transfected with IL-4R clones labelled with ^{35}S-cysteine/methionine. M1 and M2 were then injected into the peritoneal cavities of nude mice to produce ascites containing high concentrations (>1 mg/ml) of anti-IL-4R monoclonal antibody. The resulting monoclonal antibody was purified by ammonium sulfate precipitation followed by gel exclusion chromatography, and/or affinity chromatography based on binding of antibody to Protein G.

Example 14

Use of soluble IL-4R to suppress immune response *in vivo*

Experiments were conducted to determine the effect of soluble IL-4R on allogeneic host versus graft (HVG) response *in vivo* using a popliteal lymph node assay. In this model mice are injected in the footpad with irradiated, allogeneic spleen cells. Irradiated, syngeneic cells are then injected into the contralateral pad. An alloreactive response occurs in the pad receiving the allogeneic cells, the extent of which can be measured by the relative increase in size and weight of the popliteal lymph node draining the site of antigen deposition.

On day 0 three BALB/C mice were injected in the footpad with irradiated, allogeneic spleen cells from c57BL/6 mice and in the contralateral footpad with irradiated, syngeneic spleen cells. On days -1, 0 and +1 three mice were injected (intraveneously on days -1 and 0, and subcutaneously on day +1) with 100 ng of purified soluble IL-4R (sIL-4R) in phosphate buffered saline, three mice were injected intraveneously with 1μg of sIL-4R, three mice were injected with 2μg of sIL-4R and three mice were injected with MSA (control). The mean difference in weight of the lymph nodes from the sites of allogeneic and syngeneic spleen cells was approximately 2.5 mg for the mice treated with MSA, 1 mg for the mice treated with 100 ng of sIL-4R, and 0.5 mg for mice treated with 1μg sIL-4R. No detectable difference in weight of lymph nodes was ascertainable for the mice treated with 2μg sIL-4R. Thus, IL-4R significantly ($p < 0.5$ in all groups, using a two-tailed T test) suppressed the *in vivo* lymphoproliferative response in a dose dependent fashion relative to control mice.

CLAIMS

1. An isolated DNA sequence encoding a mammalian IL-4 receptor (IL-4R).

2. An isolated DNA sequence encoding a polypeptide product of procaryotic or eucaryotic host expression, said product having all or part of the primary structural conformation of a mammalian IL-4R and a biological activity of a mammalian IL-4R.

3. A DNA sequence according to claim 1, selected from the group consisting of:

(a) cDNA clones having a nucleotide sequence derived from the coding region of a native mammalian IL-4R gene;

(b) DNA sequences capable of hybridization to the clones of (a) under moderately stringent conditions and which encode biologically active IL-4R; and

(c) DNA sequences which are degenerate as a result of the genetic code to the DNA sequences defined in (a) and (b) and which encode biologically active IL-4R.

4. A DNA sequence according to claim 1, which encodes an amino acid sequence which is substantially similar to all or part of the sequence of amino acid residues 1-207 depicted in Figure 4A.

5. A DNA sequence according to claim 4, which encodes an amino acid sequence which is substantially identical to all or part of the sequence of amino acid residues 1-207 depicted in Figure 4A.

6. A recombinant expression vector comprising a DNA sequence according to any of claims 1-5.

7. A process for preparing a mammalian IL-4 receptor or an analog thereof, comprising culturing a suitable host cell comprising a vector according to claim 6 under conditions promoting expression.

8. A process for preparing a human IL-4 receptor or an analog thereof, comprising culturing a suitable host cell comprising a vector according to claim 6 under conditions promoting expression.

9. A population of eukaryotic cells which express more than 10^4 surface IL-4 receptors per cell.

10. A population of eukaryotic cells according to claim 9, which express more than 10^5 surface IL-4 receptors per cell.

11. A homogeneous biologically active mammalian IL-4 receptor composition.

12. A homogeneous biologically active mammalian IL-4 receptor composition according to claim 11, consisting essentially of murine IL-4 receptor.

13. A homogeneous biologically active mammalian IL-4 receptor composition according to claim 11, consisting essentially of human IL-4 receptor.

14. A human IL-4 receptor composition according to claim 13, wherein the IL-4 receptor is in the form of a glycoprotein having a molecular weight of between about 110,000 and 150,000 M_r by SDS-PAGE and a binding affinity (K_a) for human IL-4 of from about 1-8 x 10^9 M^{-1}.

15. A human IL-4 receptor composition according to claim 14, wherein the IL-4 receptor has an N terminal amino acid sequence Lys-Val-Leu-Gln-Glu-Pro-Thr-Cys-Val-Ser-Asp-Tyr-Met-Ser-Ile-Ser-Thr-Cys-Glu-Trp.

16. A human IL-4 receptor composition according to claim 13, wherein the transmembrane region and cytoplasmic domain of the native receptor have been deleted.

17. A human IL-4 receptor composition according to claim 13, which is greater than about 80 percent similar to all or part of the sequence of amino acid residues 1-800 depicted in Figures 4A, 4B and 4C.

18. A human IL-4 receptor composition according to claim 13, which is greater than about 80 percent similar to all or part of the sequence of amino acid residues 1-207 depicted in Figure 4A.

19. A composition for regulating immune responses in a mammal, comprising an effective amount of a composition according to claim 11, and a suitable diluent or carrier.

20. A composition accordingly to claim 19 having a specific binding activity of at least about 0.01 nanomole IL-4/nanomole IL-4 receptor.

21. A composition according to claim 19, consisting essentially of a substantially homogeneous protein composition comprising human IL-4 receptor in the form of a glycoprotein having a binding affinity (K_a) for human IL-4 of about 1-8 x 10^9 M^{-1}, and the N-terminal amino acid sequence Lys-Val-Leu-Gln-Glu-Pro-Thr-Cys-Val-Ser-Asp-Tyr-Met-Ser-Ile-Ser-Thr-Cys-Glu-Trp.

22. A homogeneous biologically active IL-4 receptor composition according to claim 11, wherein said IL-4 receptor is capable of retaining IL-4 binding activity when bound to nitrocellulose.

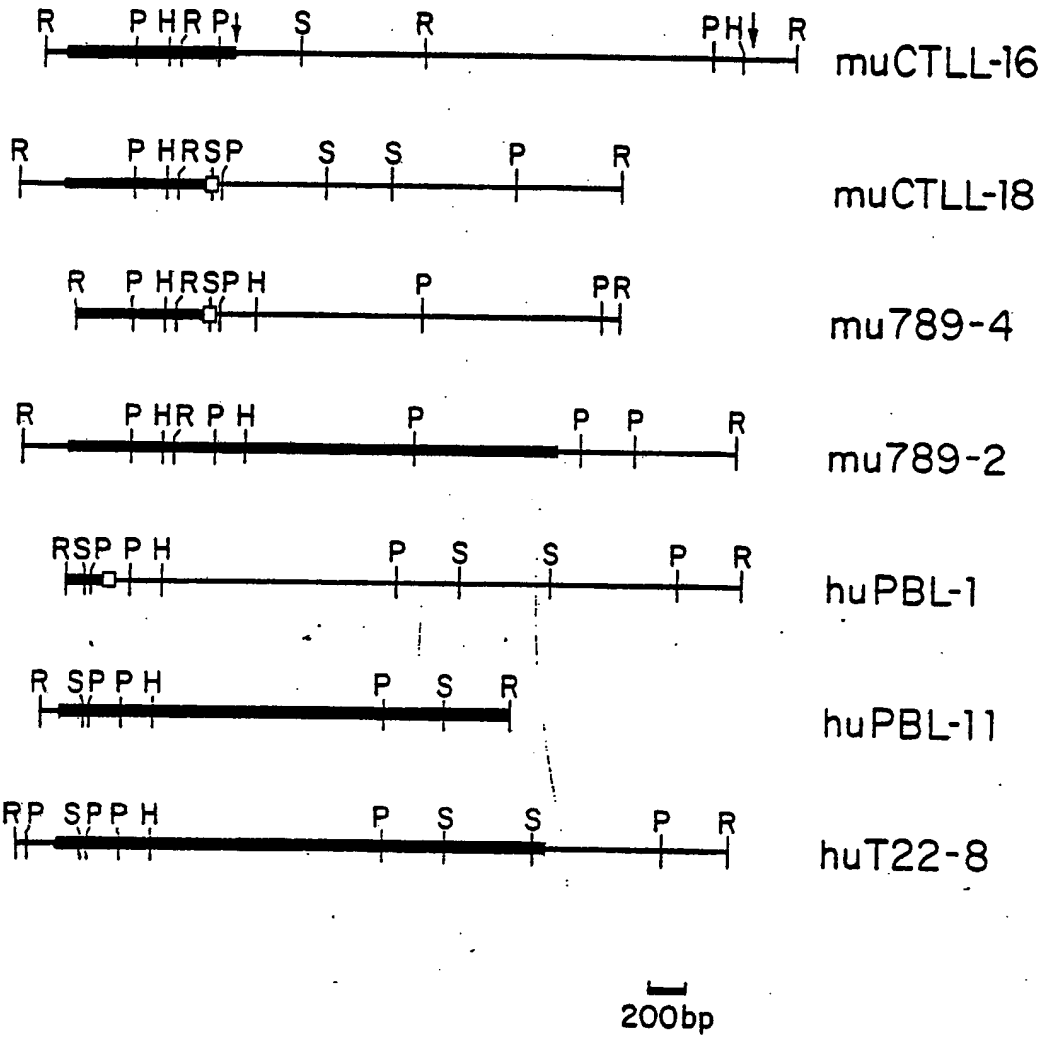
23. An assay method for detection of IL-4 or IL-4 receptor molecules or the interaction thereof, comprising use of a protein composition according to claim 19.

24. Antibodies immunoreactive with mammalian IL-4 receptors.

25. A mammalian IL-4 receptor for use in human or veterinary medicine.

26. The use of IL-4 receptor in preparing a medicament for regulating immune responses in a mammal.

27. The use of claim 26, wherein the IL-4 receptor is human IL-4 receptor and the mammal to be treated is a human.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mu789-2
R S P P H P S S P R
huPBL-1
R S P P H P S R
huPBL-11
R P S P P H P S S P R
huT22-8
200bp

Figure 1

FIGURE 2A

```
ATG GGG CGG CTT TGC ACC AAG TTC CTG ACC TCT GTG GGC TGT CTG   -31
Met Gly Arg Leu Cys Thr Lys Phe Leu Thr Ser Val Gly Cys Leu   -11

ATT TTG CTG TTG GTG ACT GGA TCT GGG AGC ATC AAG GTC CTG GGT    15
Ile Leu Leu Leu Val Thr Gly Ser Gly Ser Ile Lys Val Leu Gly     5

GAG CCC ACC TGC TTC TCT GAC TAC ATC CGC ACT TCC ACG TGT GAG    60
Glu Pro Thr Cys Phe Ser Asp Tyr Ile Arg Thr Ser Thr Cys Glu    20

TGG TTC CTG GAT AGC GCT GTG GAC TGC AGT TCT CAG CTC TGC CTA   105
Trp Phe Leu Asp Ser Ala Val Asp Cys Ser Ser Gln Leu Cys Leu    35

CAC TAC AGG CTG ATG TTC TTC GAG TTC TCT GAA AAC CTC ACA TGC   150
His Tyr Arg Leu Met Phe Phe Glu Phe Ser Glu Asn Leu Thr Cys    50

ATC CCG AGG AAC AGT GCC AGC ACT GTG TGT GTG TGC CAC ATG GAA   195
Ile Pro Arg Asn Ser Ala Ser Thr Val Cys Val Cys His Met Glu    65

ATG AAT AGG CCG GTC CAA TCA GAC AGA TAC CAG ATG GAA CTG TGG   240
Met Asn Arg Pro Val Gln Ser Asp Arg Tyr Gln Met Glu Leu Trp    80

GCT GAG CAC AGA CAG CTG TGG CAG GGC TCC TTC AGC CCC AGT GGT   285
Ala Glu His Arg Gln Leu Trp Gln Gly Ser Phe Ser Pro Ser Gly    95

AAT GTG AAG CCC CTA GCT CCA GAC AAC CTC ACA CTC CAC ACC AAT   330
Asn Val Lys Pro Leu Ala Pro Asp Asn Leu Thr Leu His Thr Asn   110

GTG TCC GAC GAA TGG CTG CTG ACC TGG AAT AAC CTG TAC CCA TCG   375
Val Ser Asp Glu Trp Leu Leu Thr Trp Asn Asn Leu Tyr Pro Ser   125

AAC AAC TTA CTG TAC AAA GAC CTC ATC TCC ATG GTC AAC ATC TCC   420
Asn Asn Leu Leu Tyr Lys Asp Leu Ile Ser Met Val Asn Ile Ser   140

AGA GAG GAC AAC CCT GCA GAA TTC ATA GTC TAT AAT GTG ACC TAC   465
Arg Glu Asp Asn Pro Ala Glu Phe Ile Val Tyr Asn Val Thr Tyr   155

AAG GAA CCC AGG CTG AGC TTC CCG ATC AAC ATC CTG ATG TCA GGG   510
Lys Glu Pro Arg Leu Ser Phe Pro Ile Asn Ile Leu Met Ser Gly   170

GTC TAC TAT ACG GCG CGT GTG AGG GTC AGA TCC CAG ATA CTC ACT   555
Val Tyr Tyr Thr Ala Arg Val Arg Val Arg Ser Gln Ile Leu Thr   185

GGC ACC TGG AGT GAG TGG AGT CCT AGC ATC ACG TGG TAC AAC CAC   600
Gly Thr Trp Ser Glu Trp Ser Pro Ser Ile Thr Trp Tyr Asn His   200

TTC CAG CTG CCC CTG ATA CAG CGC CTT CCA CTG GGG GTC ACC ATC   645
Phe Gln Leu Pro Leu Ile Gln Arg Leu Pro Leu Gly Val Thr Ile   215

TCC TGC CTC TGC ATC CCG TTG TTT TGC CTG TTC TGT TAC TTC AGC   690
Ser Cys Leu Cys Ile Pro Leu Phe Cys Leu Phe Cys Tyr Phe Ser   230

ATT ACC AAG ATT AAG AAG ATA TGG TGG GAC CAG ATT CCC ACC CCA   735
Ile Thr Lys Ile Lys Lys Ile Trp Trp Asp Gln Ile Pro Thr Pro   245
```

FIGURE 2B

```
GCA CGC AGT CCC TTG GTG GCC ATC ATC ATT CAG GAT GCA CAG GTG   780
Ala Arg Ser Pro Leu Val Ala Ile Ile Ile Gln Asp Ala Gln Val   260

CCC CTC TGG GAT AAG CAG ACC CGA AGC CAG GAG TCA ACC AAG TAC   825
Pro Leu Trp Asp Lys Gln Thr Arg Ser Gln Glu Ser Thr Lys Tyr   275

CCG CAC TGG AAA ACT TGT CTA GAC AAG CTG CTG CCT TGC TTG CTG   870
Pro His Trp Lys Thr Cys Leu Asp Lys Leu Leu Pro Cys Leu Leu   290

AAG CAC AGA GTA AAG AAG AAG ACA GAC TTC CCG AAG GCT GCC CCA   915
Lys His Arg Val Lys Lys Lys Thr Asp Phe Pro Lys Ala Ala Pro   305

ACC AAG TCT CTC CAG AGT CCT GGA AAG GCA GGC TGG TGT CCC ATG   960
Thr Lys Ser Leu Gln Ser Pro Gly Lys Ala Gly Trp Cys Pro Met   320

GAG GTC AGC AGG ACC GTC CTC TGG CCA GAG AAT GTT AGT GTC AGT  1005
Glu Val Ser Arg Thr Val Leu Trp Pro Glu Asn Val Ser Val Ser   335

GTG GTG CGC TGT ATG GAG CTG TTT GAG GCC CCA GTA CAG AAT GTG  1050
Val Val Arg Cys Met Glu Leu Phe Glu Ala Pro Val Gln Asn Val   350

GAG GAG GAA GAA GAT GAG ATA GTC AAA GAG GAC CTG AGC ATG TCA  1095
Glu Glu Glu Glu Asp Glu Ile Val Lys Glu Asp Leu Ser Met Ser   365

CCT GAG AAC AGC GGA GGC TGC GGC TTC CAG GAG aGC CAG GCA GAC  1140
Pro Glu Asn Ser Gly Gly Cys Gly Phe Gln Glu Ser Gln Ala Asp   380

ATC ATG GCT CGG CTC ACT GAG AAC CTG TTT TCC GAC TTG TTG GAG  1185
Ile Met Ala Arg Leu Thr Glu Asn Leu Phe Ser Asp Leu Leu Glu   395

GCT GAG AAT GGG GGC CTT GGC CAG TCA GCC TTG GCA GAG TCA TGC  1230
Ala Glu Asn Gly Gly Leu Gly Gln Ser Ala Leu Ala Glu Ser Cys   410

TCC CCT CTG CCT TCA GGA AGT GGG CAG GCT TCT GTA TCC TGG GCC  1275
Ser Pro Leu Pro Ser Gly Ser Gly Gln Ala Ser Val Ser Trp Ala   425

TGC CTC CCC ATG GGG CCC AGT GAG GAG GCC ACA TGC CAG GTC ACA  1320
Cys Leu Pro Met Gly Pro Ser Glu Glu Ala Thr Cys Gln Val Thr   440

GAG CAG CCT TCA CAC CCA GGC CCT CTT TCA GGC AGC CCA GCC CAG  1365
Glu Gln Pro Ser His Pro Gly Pro Leu Ser Gly Ser Pro Ala Gln   455

AGT GCA CCT ACT CTG GCT TGC ACG CAG GTC CCA CTT GTC CTT GCA  1410
Ser Ala Pro Thr Leu Ala Cys Thr Gln Val Pro Leu Val Leu Ala   470

GAC AAT CCT GCC TAC CGG AGT TTT AGT GAC TGC TGT AGC CCG GCC  1455
Asp Asn Pro Ala Tyr Arg Ser Phe Ser Asp Cys Cys Ser Pro Ala   485

CCA AAT CCT GGA GAG CTG GCT CCA GAG CAG CAG CAG GCT GAT CAT  1500
Pro Asn Pro Gly Glu Leu Ala Pro Glu Gln Gln Gln Ala Asp His   500

CTG GAA GAA GAG GAG CCT CCA AGC CCG GCT GAC CCC CAT TCT TCA  1545
Leu Glu Glu Glu Glu Pro Pro Ser Pro Ala Asp Pro His Ser Ser   515
```

FIGURE 2C

```
GGG CCA CCA ATG CAG CCA GTG GAG AGC TGG GAG CAG ATC CTT CAC  1590
Gly Pro Pro Met Gln Pro Val Glu Ser Trp Glu Gln Ile Leu His   530

ATG AGT GTC CTG CAG CAT GGG GCA GCT GCT GGC TCC ACC CCA GCC  1635
Met Ser Val Leu Gln His Gly Ala Ala Ala Gly Ser Thr Pro Ala   545

CCT GCC GGT GGC TAC CAG GAG TTT GTG CAG GCA GTG AAG CAG GGT  1680
Pro Ala Gly Gly Tyr Gln Glu Phe Val Gln Ala Val Lys Gln Gly   560

GCC GCC CAG GAT CCT GGG GTG CCT GGT GTC AGG CCT TCT GGA GAC  1725
Ala Ala Gln Asp Pro Gly Val Pro Gly Val Arg Pro Ser Gly Asp   575

CCC GGT TAC AAG GCC TTC TCG AGC CTG CTC AGC AGC AAT GGC ATC  1770
Pro Gly Tyr Lys Ala Phe Ser Ser Leu Leu Ser Ser Asn Gly Ile   590

CGC GGG GAC ACA GCA GCA GCG GGG ACT GAC GAT GGG CAT GGA GGC  1815
Arg Gly Asp Thr Ala Ala Ala Gly Thr Asp Asp Gly His Gly Gly   605

TAC AAG CCC TTC CAG AAT CCT GTT CCT AAC CAG TCC CCT AGC TCC  1860
Tyr Lys Pro Phe Gln Asn Pro Val Pro Asn Gln Ser Pro Ser Ser   620

GTG CCC TTA TTT ACT TTC GGA CTA GAC ACG GAG CTG TCA CCC AGT  1905
Val Pro Leu Phe Thr Phe Gly Leu Asp Thr Glu Leu Ser Pro Ser   635

CCT CTG AAC TCA GAC CCA CCC AAA AGC CCC CCA GAA TGC CTT GGT  1950
Pro Leu Asn Ser Asp Pro Pro Lys Ser Pro Pro Glu Cys Leu Gly   650

CTG GAG CTG GGG CTC AAA GGA GGT GAC TGG GTG AAG GCC CCT CCT  1995
Leu Glu Leu Gly Leu Lys Gly Gly Asp Trp Val Lys Ala Pro Pro   665

CCT GCA GAT GAG GTG CCC AAG CCC TTT GGG GAT GAC CTG GGC TTT  2040
Pro Ala Asp Glu Val Pro Lys Pro Phe Gly Asp Asp Leu Gly Phe   680

GGT ATT GTG TAC TCG TCC CTC ACT TGC CAC TTG TGT GGC CAC CTG  2085
Gly Ile Val Tyr Ser Ser Leu Thr Cys His Leu Cys Gly His Leu   695

AAG CAA CAC CAC AGC CAG GAG GAA GGT GGC CAG AGC CCC ATC GTT  2130
Lys Gln His His Ser Gln Glu Glu Gly Gly Gln Ser Pro Ile Val   710

GCT AGC CCT GGC TGT GGC TGC TGC TAC GAT GAC AGA TCA CCA TCC  2175
Ala Ser Pro Gly Cys Gly Cys Cys Tyr Asp Asp Arg Ser Pro Ser   725

CTG GGG AGC CTC TCG GGG GCC TTG GAA AGC TGT CCT GAG GGA ATA  2220
Leu Gly Ser Leu Ser Gly Ala Leu Glu Ser Cys Pro Glu Gly Ile   740

CCA CCA GAA GCC AAC CTC ATG TCA GCA CCC AAG ACA CCC TCA AAC  2265
Pro Pro Glu Ala Asn Leu Met Ser Ala Pro Lys Thr Pro Ser Asn   755

TTG TCA GGG GAG GGC AAG GGC CCT GGT CAC TCT CCT GTT CCC AGC  2310
Leu Ser Gly Glu Gly Lys Gly Pro Gly His Ser Pro Val Pro Ser   770

CAG ACG ACC GAG GTG CCT GTG GGC GCC CTG GGC ATT GCT GTT TCT  2355
Gln Thr Thr Glu Val Pro Val Gly Ala Leu Gly Ile Ala Val Ser   785
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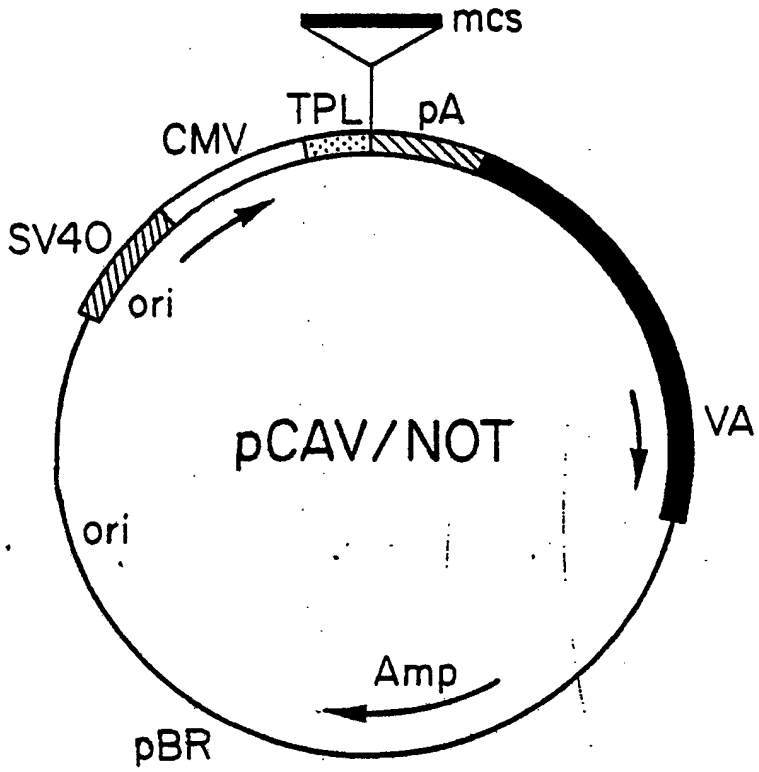

Figure 3

FIGURE 4A

```
ATG GGG TGG CTT TGC TCT GGG CTC CTG TTC CCT GTG AGC TGC CTG   -31
Met Gly Trp Leu Cys Ser Gly Leu Leu Phe Pro Val Ser Cys Leu   -11

GTC CTG CTG CAG GTG GCA AGC TCT GGG AAC ATG AAG GTC TTG CAG    15
Val Leu Leu Gln Val Ala Ser Ser Gly Asn Met Lys Val Leu Gln     5

GAG CCC ACC TGC GTC TCC GAC TAC ATG AGC ATC TCT ACT TGC GAG    60
Glu Pro Thr Cys Val Ser Asp Tyr Met Ser Ile Ser Thr Cys Glu    20

TGG AAG ATG AAT GGT CCC ACC AAT TGC AGC ACC GAG CTC CGC CTG   105
Trp Lys Met Asn Gly Pro Thr Asn Cys Ser Thr Glu Leu Arg Leu    35

TTG TAC CAG CTG GTT TTT CTG CTC TCC GAA GCC CAC ACG TGT ATC   150
Leu Tyr Gln Leu Val Phe Leu Leu Ser Glu Ala His Thr Cys Ile    50

CCT GAG AAC AAC GGA GGC GCG GGG TGC GTG TGC CAC CTG CTC ATG   195
Pro Glu Asn Asn Gly Gly Ala Gly Cys Val Cys His Leu Leu Met    65

GAT GAC GTG GTC AGT GCG GAT AAC TAT ACA CTG GAC CTG TGG GCT   240
Asp Asp Val Val Ser Ala Asp Asn Tyr Thr Leu Asp Leu Trp Ala    80

GGG CAG CAG CTG CTG TGG AAG GGC TCC TTC AAG CCC AGC GAG CAT   285
Gly Gln Gln Leu Leu Trp Lys Gly Ser Phe Lys Pro Ser Glu His    95

GTG AAA CCC AGG GCC CCA GGA AAC CTG ACA GTT CAC ACC AAT GTC   330
Val Lys Pro Arg Ala Pro Gly Asn Leu Thr Val His Thr Asn Val   110

TCC GAC ACT CTG CTG CTG ACC TGG AGC AAC CCG TAT CCC CCT GAC   375
Ser Asp Thr Leu Leu Leu Thr Trp Ser Asn Pro Tyr Pro Pro Asp   125

AAT TAC CTG TAT AAT CAT CTC ACC TAT GCA GTC AAC ATT TGG AGT   420
Asn Tyr Leu Tyr Asn His Leu Thr Tyr Ala Val Asn Ile Trp Ser   140

GAA AAC GAC CCG GCA GAT TTC AGA ATC TAT AAC GTG ACC TAC CTA   465
Glu Asn Asp Pro Ala Asp Phe Arg Ile Tyr Asn Val Thr Tyr Leu   155

GAA CCC TCC CTC CGC ATC GCA GCC AGC ACC CTG AAG TCT GGG ATT   510
Glu Pro Ser Leu Arg Ile Ala Ala Ser Thr Leu Lys Ser Gly Ile   170

TCC TAC AGG GCA CGG GTG AGG GCC TGG GCT CAG TGC TAT AAC ACC   555
Ser Tyr Arg Ala Arg Val Arg Ala Trp Ala Gln Cys Tyr Asn Thr   185

ACC TGG AGT GAG TGG AGC CCC AGC ACC AAG TGG CAC AAC TCC TAC   600
Thr Trp Ser Glu Trp Ser Pro Ser Thr Lys Trp His Asn Ser Tyr   200

AGG GAG CCC TTC GAG CAG CAC CTC CTG CTG GGC GTC AGC GTT TCC   645
Arg Glu Pro Phe Glu Gln His Leu Leu Leu Gly Val Ser Val Ser   215

TGC ATT GTC ATC CTG GCC GTC TGC CTG TTG TGC TAT GTC AGC ATC   690
Cys Ile Val Ile Leu Ala Val Cys Leu Leu Cys Tyr Val Ser Ile   230

ACC AAG ATT AAG AAA GAA TGG TGG GAT CAG ATT CCC AAC CCA GCC   735
Thr Lys Ile Lys Lys Glu Trp Trp Asp Gln Ile Pro Asn Pro Ala   245
```

FIGURE 4B

```
CGC AGC CGC CTC GTG GCT ATA ATA ATC CAG GAT GCT CAG GGG TCA  780
Arg Ser Arg Leu Val Ala Ile Ile Ile Gln Asp Ala Gln Gly Ser  260

CAG TGG GAG AAG CGG TCC CGA GGC CAG GAA CCA GCC AAG TGC CCA  825
Gln Trp Glu Lys Arg Ser Arg Gly Gln Glu Pro Ala Lys Cys Pro  275

CAC TGG AAG AAT TGT CTT ACC AAG CTC TTG CCC TGT TTT CTG GAG  870
His Trp Lys Asn Cys Leu Thr Lys Leu Leu Pro Cys Phe Leu Glu  290

CAC AAC ATG AAA AGG GAT GAA GAT CCT CAC AAG GCT GCC AAA GAG  915
His Asn Met Lys Arg Asp Glu Asp Pro His Lys Ala Ala Lys Glu  305

ATG CCT TTC CAG GGC TCT GGA AAA TCA GCA TGG TGC CCA GTG GAG  960
Met Pro Phe Gln Gly Ser Gly Lys Ser Ala Trp Cys Pro Val Glu  320

ATC AGC AAG ACA GTC CTC TGG CCA GAG AGC ATC AGC GTG GTG CGA 1005
Ile Ser Lys Thr Val Leu Trp Pro Glu Ser Ile Ser Val Val Arg  335

TGT GTG GAG TTG TTT GAG GCC CCG GTG GAG TGT GAG GAG GAG GAG 1050
Cys Val Glu Leu Phe Glu Ala Pro Val Glu Cys Glu Glu Glu Glu  350

GAG GTA GAG GAA GAA AAA GGG AGC TTC TGT GCA TCG CCT GAG AGC 1095
Glu Val Glu Glu Glu Lys Gly Ser Phe Cys Ala Ser Pro Glu Ser  365

AGC AGG GAT GAC TTC CAG GAG GGA AGG GAG GGC ATT GTG GCC CGG 1140
Ser Arg Asp Asp Phe Gln Glu Gly Arg Glu Gly Ile Val Ala Arg  380

CTA ACA GAG AGC CTG TTC CTG GAC CTG CTC GGA GAG GAG AAT GGG 1185
Leu Thr Glu Ser Leu Phe Leu Asp Leu Leu Gly Glu Glu Asn Gly  395

GGC TTT TGC CAG CAG GAC ATG GGG GAG TCA TGC CTT CTT CCA CCT 1230
Gly Phe Cys Gln Gln Asp Met Gly Glu Ser Cys Leu Leu Pro Pro  410

TCG GGA AGT ACG AGT GCT CAC ATG CCC TGG GAT GAG TTC CCA AGT 1275
Ser Gly Ser Thr Ser Ala His Met Pro Trp Asp Glu Phe Pro Ser  425

GCA GGG CCC AAG GAG GCA CCT CCC TGG GGC AAG GAG CAG CCT CTC 1320
Ala Gly Pro Lys Glu Ala Pro Pro Trp Gly Lys Glu Gln Pro Leu  440

CAC CTG GAG CCA AGT CCT CCT GCC AGC CCG ACC CAG AGT CCA GAC 1365
His Leu Glu Pro Ser Pro Pro Ala Ser Pro Thr Gln Ser Pro Asp  455

AAC CTG ACT TGC ACA GAG ACG CCC CTC GTC ATC GCA GGC AAC CCT 1410
Asn Leu Thr Cys Thr Glu Thr Pro Leu Val Ile Ala Gly Asn Pro  470

GCT TAC CGC AGC TTC AGC AAC TCC CTG AGC CAG TCA CCG TGT CCC 1455
Ala Tyr Arg Ser Phe Ser Asn Ser Leu Ser Gln Ser Pro Cys Pro  485

AGA GAG CTG GGT CCA GAC CCA CTG CTG GCC AGA CAC CTG GAG GAA 1500
Arg Glu Leu Gly Pro Asp Pro Leu Leu Ala Arg His Leu Glu Glu  500

GTA GAA CCC GAG ATG CCC TGT GTC CCC CAG CTC TCT GAG CCA ACC 1545
Val Glu Pro Glu Met Pro Cys Val Pro Gln Leu Ser Glu Pro Thr  515
```

FIGURE 4C

```
ACT GTG CCC CAA CCT GAG CCA GAA ACC TGG GAG CAG ATC CTC CGC 1590
Thr Val Pro Gln Pro Glu Pro Glu Thr Trp Glu Gln Ile Leu Arg  530

CGA AAT GTC CTC CAG CAT GGG GCA GCT GCA GCC CCC GTC TCG GCC 1635
Arg Asn Val Leu Gln His Gly Ala Ala Ala Ala Pro Val Ser Ala  545

CCC ACC AGT GGC TAT CAG GAG TTT GTA CAT GCG GTG GAG CAG GGT 1680
Pro Thr Ser Gly Tyr Gln Glu Phe Val His Ala Val Glu Gln Gly  560

GGC ACC CAG GCC AGT GCG GTG GTG GGC TTG GGT CCC CCA GGA GAG 1725
Gly Thr Gln Ala Ser Ala Val Val Gly Leu Gly Pro Pro Gly Glu  575

GCT GGT TAC AAG GCC TTC TCA AGC CTG CTT GCC AGC AGT GCT GTG 1770
Ala Gly Tyr Lys Ala Phe Ser Ser Leu Leu Ala Ser Ser Ala Val  590

TCC CCA GAG AAA TGT GGG TTT GGG GCT AGC AGT GGG GAA GAG GGG 1815
Ser Pro Glu Lys Cys Gly Phe Gly Ala Ser Ser Gly Glu Glu Gly  605

TAT AAG CCT TTC CAA GAC CTC ATT CCT GGC TGC CCT GGG GAC CCT 1860
Tyr Lys Pro Phe Gln Asp Leu Ile Pro Gly Cys Pro Gly Asp Pro  620

GCC CCA GTC CCT GTC CCC TTG TTC ACC TTT GGA CTG GAC AGG GAG 1905
Ala Pro Val Pro Val Pro Leu Phe Thr Phe Gly Leu Asp Arg Glu  635

CCA CCT CGC AGT CCG CAG AGC TCA CAT CTC CCA AGC AGC TCC CCA 1950
Pro Pro Arg Ser Pro Gln Ser Ser His Leu Pro Ser Ser Ser Pro  650

GAG CAC CTG GGT CTG GAG CCG GGG GAA AAG GTA GAG GAC ATG CCA 1995
Glu His Leu Gly Leu Glu Pro Gly Glu Lys Val Glu Asp Met Pro  665

AAG CCC CCA CTT CCC CAG GAG CAG GCC ACA GAC CCC CTT GTG GAC 2040
Lys Pro Pro Leu Pro Gln Glu Gln Ala Thr Asp Pro Leu Val Asp  680

AGC CTG GGC AGT GGC ATT GTC TAC TCA GCC CTT ACC TGC CAC CTG 2085
Ser Leu Gly Ser Gly Ile Val Tyr Ser Ala Leu Thr Cys His Leu  695

TGC GGC CAC CTG AAA CAG TGT CAT GGC CAG GAG GAT GGT GGC CAG 2130
Cys Gly His Leu Lys Gln Cys His Gly Gln Glu Asp Gly Gly Gln  710

ACC CCT GTC ATG GCC AGT CCT TGC TGT GGC TGC TGC TGT GGA GAC 2175
Thr Pro Val Met Ala Ser Pro Cys Cys Gly Cys Cys Cys Gly Asp  725

AGG TCC TCG CCC CCT ACA ACC CCC CTG AGG GCC CCA GAC CCC TCT 2220
Arg Ser Ser Pro Pro Thr Thr Pro Leu Arg Ala Pro Asp Pro Ser  740

CCA GGT GGG GTT CCA CTG GAG GCC AGT CTG TGT CCG GCC TCC CTG 2265
Pro Gly Gly Val Pro Leu Glu Ala Ser Leu Cys Pro Ala Ser Leu  755

GCA CCC TCG GGC ATC TCA GAG AAG AGT AAA TCC TCA TCA TCC TTC 2310
Ala Pro Ser Gly Ile Ser Glu Lys Ser Lys Ser Ser Ser Ser Phe  770

CAT CCT GCC CCT GGC AAT GCT CAG AGC TCA AGC CAG ACC CCC AAA 2355
His Pro Ala Pro Gly Asn Ala Gln Ser Ser Ser Gln Thr Pro Lys  785

ATC GTG AAC TTT GTC TCC GTG GGA CCC ACA TAC ATG AGG GTC TCT 2400
Ile Val Asn Phe Val Ser Val Gly Pro Thr Tyr Met Arg Val Ser  800
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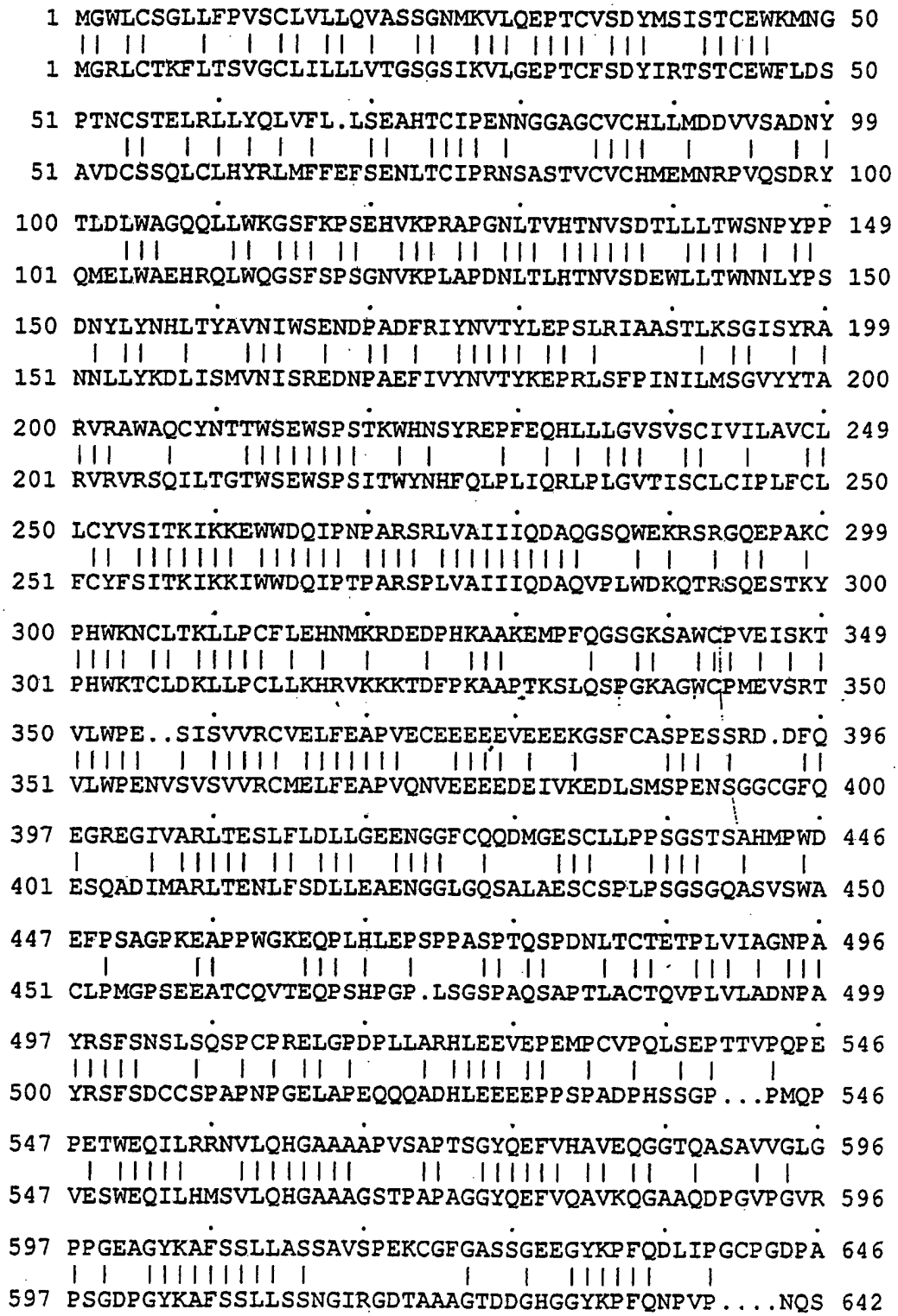
FIGURE 5A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100 TLDLWAGQQLLWKGSFKPSEHVKPRAPGNLTVHTNVSDTLLLTWSNPYPP 149
101 QMELWAEHRQLWQGSFSPSGNVKPLAPDNLTLHTNVSDEWLLTWNNLYPS 150
150 DNYLYNHLTYAVNIWSENDPADFRIYNVTYLEPSLRIAASTLKSGISYRA 199
151 NNLLYKDLISMVNISREDNPAEFIVYNVTYKEPRLSFPINILMSGVYYTA 200
200 RVRAWAQCYNTTWSEWSPSTKWHNSYREPFEQHLLLGVSVSCIVILAVCL 249
201 RVRVRSQILTGTWSEWSPSITWYNHFQLPLIQRLPLGVTISCLCIPLFCL 250
250 LCYVSITKIKKEWWDQIPNPARSRLVAIIIQDAQGSQWEKRSRGQEPAKC 299
251 FCYFSITKIKKIWWDQIPTPARSPLVAIIIQDAQVPLWDKQTRSQESTKY 300
300 PHWKNCLTKLLPCFLEHNMKRDEDPHKAAKEMPFQGSGKSAWCPVEISKT 349
301 PHWKTCLDKLLPCLLKHRVKKKTDFPKAAPTKSLQSPGKAGWCPMEVSRT 350
350 VLWPE..SISVVRCVELFEAPVECEEEEEVEEEKGSFCASPESSRD.DFQ 396
351 VLWPENVSVSVVRCMELFEAPVQNVEEEEDEIVKEDLSMSPENSGGCGFQ 400
397 EGREGIVARLTESLFLDLLGEENGGFCQQDMGESCLLPPSGSTSAHMPWD 446
401 ESQADIMARLTENLFSDLLEAENGGLGQSALAESCSPLPSGSGQASVSWA 450
447 EFPSAGPKEAPPWGKEQPLHLEPSPPASPTQSPDNLTCTETPLVIAGNPA 496
451 CLPMGPSEEATCQVTEQPSHPGP.LSGSPAQSAPTLACTQVPLVLADNPA 499
497 YRSFSNSLSQSPCPRELGPDPLLARHLEEVEPEMPCVPQLSEPTTVPQPE 546
500 YRSFSDCCSPAPNPGELAPEQQQADHLEEEEPPSPADPHSSGP...PMQP 546
547 PETWEQILRRNVLQHGAAAAPVSAPTSGYQEFVHAVEQGGTQASAVVGLG 596
547 VESWEQILHMSVLQHGAAAGSTPAPAGGYQEFVQAVKQGAAQDPGVPGVR 596
597 PPGEAGYKAFSSLLASSAVSPEKCGFGASSGEEGYKPFQDLIPGCPGDPA 646
597 PSGDPGYKAFSSLLSSNGIRGDTAAAGTDDGHGGYKPFQNPVP....NQS 642

FIGURE 5B

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647 PVPVPLFTFGLDREPPRSPQSSHLPSSSPEHLGLEPGEKVEDMPKPPLPQ 696
    |  |||||||||| |   ||  |  | | || |||| | |  |  | | |
643 PSSVPLFTFGLDTELSPSPLNSDPPKSPPECLGLELGLKGGDWVKAPPPA 692

697 EQATDPLVDSLGSGIVYSALTCHLCGHLKQCHGQEDGGQTPVMASPCCGC 746
     |   |  | || ||||| |||||||||||| | || ||| |  ||| |||
693 DQVPKPFGDDLGFGIVYSSLTCHLCGHLKQHHSQEEGGQSPIVASPGCGC 742

747 CCGDRSSPPTTPLRAPDPSPGGVPLEASLCPASLAPSGISEKSKSSSSFH 796
    |  |||         |    | | | || |  |   ||  |   |
743 CYDDRSPSLGSLSGALESCPEGIPPEANLMSAPKTPSNLSGEGK..... 786

797 PAPGNAQSSSQTPKIVNFVSVGPTYMRVS 825
      ||     |||      | ||     ||
787 .GPGHSPVPSQTTE....VPVGALGIAVS 810
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INTERNATIONAL SEARCH REPORT

International Application No PCT/US 89/04076

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: C 12 N 15/12, 15/62, C 12 P 21/02, A 61 K 37/02, C 07 K 13/00, C 12 P 21/08, G 01 N 33/68

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	C 12 N, C 12 P, A 61 K, C 07 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	Immunology, vol. 54, 1985, R.A. De Maagd et al.: "The human thymus microenvironment: heterogeneity detected by monoclonal anti-epithelial cell antibodies", pages 745-754, see the whole article --	24
P,X	Immunology, vol. 65, 1988, M. Larche et al.: "Functional evidence for a monoclonal antibody that binds to the human IL-4 receptor", pages 617-622, see the whole article --	24
P,X	Immunology, vol. 64, 1988, M. Larche et al.: "A novel T-lymphocyte molecule that may function in the induction of self-tolerance and MHC-restriction within the human thymic microenvironment" pages 101-105, see the whole article --	11,13,24

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* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
19th January 1990	21 FEV. 1990
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	T.K. WILLIS

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
P,X	WO, A, 89/09621 (RITTER) 19 October 1989, see the whole document --	11,13,24
A	Proc. Natl. Acad. Sci, USA, vol. 84, March 1987, (US), L.S. Park et al.: "Characterization of the high-affinity cell-surface receptor for murine B-cell-stimulating factor 1", pages 1669-1673, see the whole article --	11,13
A	Nature, vol. 325, 5 February 1987, J. Ohara et al.: "Receptors for B-cell stimulatory factor-1 expressed on cells of haematopoietic lineage" pages 537-540, see the whole article	11,13

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 8904076 SA 31825

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 09/02/90 The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A- 8909621	19-10-89	None	